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# INVESTIGATION OF EPILEPTIFORM ATTACKS PRODUCED BY SUDDEN COOLING OF FROG SPINAL CORD

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(Received for publication May 12, 1942)

In 1933 it was found that sudden cooling of the spinal cord of Brazilian frogs with cord and posterior extremities isolated, caused an epileptiform attack. No one previously had obtained attacks of this type in frogs or in other animals. Faradic stimulation was without effect; also constant current stimulation (2). In re-examining the question with various types of stimulating currents, it was determined that Brazilian frogs did not develop attacks with any type of electrical stimulation.

Detailed studies were accordingly made of cold seizures. One point hitherto unstudied is presented in this investigation, namely, that the attacks exhibit characteristics different in different species of frogs and they show considerable modifications in the same species transferred to a different climate. The influence of climate and other conditions in which the animals live has an influence on the characteristics of seizures. Also for the South American frogs (*Leptodactylus ocellatus*) the upper limit of temperature is higher at Rio, 8.5°C. than at Buenos Aires or Montevideo, 5°C., and higher still at Pernambuco 12°C. than at Rio. The form of the attack is the same at Buenos Aires and at Montevideo where climatic conditions are closely similar. It has been demonstrated also that North American frogs transported to Rio do not have attacks even at 0°, indeed one cannot even induce them by lowering the temperature several degrees below zero. The change in character of the epileptiform attacks, the differences of species and conditions of life raise interesting questions. The present study describes experiments made at Yale with North American frogs during the winter of 1942.

## TECHNIQUES

Several different procedures were followed for recording an attack by sudden cooling. In one group it was arranged to use Ringer solution so arranged as to be chilled quickly by circulating it through ice water, previously described. The isolated spinal cord preparation could be plunged quickly in a cold Ringer bath with the hind extremities attached to a cord through the sciatic nerve, rested outside the bath and arranged for myographic recordings. The temperature of the bath was recorded by a simple thermometer. When it was necessary to cool the cord below 0°C. it was still possible to use the bath method replacing the Ringer by liquid paraffin (petrolatum) or even by a concentrated sodium chloride. It has been established that at extremely low temperatures hypertonic solutions do not stimulate nerves. Another method consists of applying ether, or better, ethyl chloride, on the unopened vertebral column. It is necessary to place the preparation in a position so that the ether or ethyl chloral does not fall on the nerve trunks as they emerge from the cord. One can also evoke attacks by applying carbon dioxide snow on the vertebral column. These experiments have been used with "dry ice."

RESULTS

*Absence of attacks at 0° or below in North American frogs.* In a number of experiments performed at New Haven with North American frogs it was possible to confirm the point already observed, namely, that attacks were not caused at temperatures above 0°C. In the majority of cases there was no reaction. The muscles of the isolated hind limbs remained perfectly immobile, even though the cord were exposed to the low temperature for ten, fifteen and even twenty minutes. In several cases, although no attack was produced, there were fibrillary twitchings similar to those which precede ordinary attacks. The twitchings were feeble and usually lasted only one to two minutes.

Only once was a full attack produced, the temperature of the bath was 0.5°C., the attack tonic and clonic, long and prolonged, commenced 7.5 minutes after the beginning of the chilling. This resilient form lasts from 1 to 1.5 minutes after the cord is plunged into the bath. It is not easy to explain this single exception contrary to all our experience in other North American frogs. The animal apparently was not abnormal.

In previous experiments it was found that attacks developed without external excitation, sections of the posterior nerve roots did not prevent them. External excitation, however, did have some influence upon the attacks. Repeated stimulation of the foot pads during chilling of the cord might lead to an attack at a temperature several degrees above the superior limit at which attacks ordinarily occurred. The reflex influence was ipsilateral. In North American frogs, reflex attacks could not be demonstrated under 0° but twitching could be induced and pronounced chronic seizures did not develop.

In resumé, epileptiform attacks caused by sudden chilling of the spinal cord of North American frogs can not be caused in temperatures above 0°C.

*With cord exposed.* Experiments with Brazilian frogs had shown that the speed of chilling exerted a considerable influence on the attack. If, in place of being sudden, the chilling proceeds slowly, low temperatures may be attained without causing an attack. The opening of the vertebral canal, with the cord exposed and submitted to direct action of cold, accelerates the chilling. The latent period, that is to say, the time between the onset of action of the cold and the beginning of the attack was reduced to about 10 seconds, if the temperature of the bath is below 4°C. Otherwise, one can cause an attack up to 12°, whereas the upper limit is 8.5° and the vertebral canal remains closed.

In North American frogs it is possible to obtain a characteristic attack after exposure of the cord. The operation is performed one half hour before the experiment. Before making the preparation, the determination whether the animal's voluntary movements are normal is made, and after preparation, whether the reflexes are in good condition, and finally assurance that neither the cord nor its roots have been injured during the operation.

Under these conditions only once was there no attack. In all other ex-

periments sharp attacks occurred. The latent period varied from one to three minutes, the temperature of the cold bath being 0°–0.5°. This is a latent period much larger than that in the same conditions with *Leptodactylus ocellatus*.

The attack in *Rana pipiens* brought on by chilling after exposure of the cord, begins about one minute after the onset of chilling, by trembling fibrillations, which become stronger and stronger; there then follows a phase of disordered clonic contractions, and finally a tonic phase, the clonic contractions becoming additive from the contraction of the muscles. The attack is much more prolonged in the Brazilian frogs and in certain cases, endured for four minutes. It is almost always asymmetric, as is the case of attacks in European frogs, and contrariwise of those in *Leptodactylus ocellatus*. In one case at Yale the attack only commenced in the right leg when the convulsions disappeared in the left leg.

To sum up, the attacks were produced in North American frogs at 0°, or even a little below 0°, when the spinal column is opened and the cord is directly exposed to the action of cold.

*3. Attack produced below 0°C.* In North American frogs attacks by quick cooling of the cord is always produced, if the temperature attained is sufficiently low and below the freezing point of water. The attack is not uniform and may show divergent characteristics depending on the method employed, which is probably due to differences in the rapidity of the chilling.

The application of ether, which is applied drop by drop on the dorsal surface of the exposed cord is often sufficient to produce an attack. Ethyl chloride is, however, less efficacious. In some cases the attack is almost exclusively clonic and lasts about two minutes. The tonic phase is absent or is feeble and reduced. After the attack, the reflexes return if the cord is again placed in a Ringer bath at room temperature. The application of dry ice on the closed spinal cord is marked by the production of extraordinarily intense attacks. The latent period is brief. Some seconds after the onset of chilling clonic contractions of muscles have already been initiated. The contractions increase and a period of tonic contractions follows, tetanic in nature and of great intensity. In general the attack is not symmetrical.

If, on the one hand, application of dry ice produces the phenomenon with exceptional intensity this method still is not entirely free of objection. The chilling is too extreme and cannot be applied with comfort. In consequence of this when the attack has ended, if one rewarms the cord by removing it to the circumambient temperature, the reflexes do not return: the centers have been functionally destroyed. On the other hand, the chilling may reach the nerves. In the Ringer bath at 0°, the nerves give no reaction; they are not excited by the cold. When ethyl chloride is employed, if a drop falls on the nerve, there are some quick muscular contractions and the cord loses its conductibility, but it is always possible to distinguish among the contractions those which are of central origin. With dry ice, chilling of the nerves across the other tissues may by itself give fairly strong contractions of the muscles.

The experiments of control are made with preparations in which the cord has been previously at the interior of the vertebral canal. However, the application of dry ice on the dorsal surface of the spinal cord gives place to contractions of the muscles of the legs. These contractions due to chilling of the nerves through the other tissues begin much later than in the case of the attack; their duration is much more reduced, they are much less intense, not presenting a tonic phase and not showing a systematization like that observed in a true attack.

The dry ice can produce such attacks in the whole body without the necessity of isolating the vertebral column. Thus in many experiments the cord was separated from the superior centers and the skin of the back elevated. The frog was placed on the edge of the table so that the posterior limbs remained free. The application of the dry ice on the back gave rise to violent attacks, with convulsions not only of the posterior, but of the anterior members and of the muscles of the body. The latent period is long. The reflexes did not return after the reheating, except in one case and then only in the anterior members.

When paraffin oil is employed as a bath, it is necessary to reduce the temperature extremely low in order to obtain an attack and that is probably due to the reduced calorific conductability of the oil. At 6° below no attack is produced. At 18° below the attack is feeble and entirely clonic.

In a bath of concentrated solution of NaCl, chilled below 0°, the attack is strong and presents a tonic phase.

4. *Means of inducing an attack at 0° or below 0°.* It is possible to obtain an attack at 0°, or even below 0°, without exposing the cord by opening the vertebral canal. The first method consists of injecting a quarter of an hour before the preparation a certain dose of caffeine in every case insufficient for producing visible intoxication. Good results were obtained with one injection of 1 or 1.5 cc. of a solution of benzoate of caffeine in the lymphatic sac. The attack is produced up to 3.5°, showing fairly strong clonic and tonic contractions and being prolonged for 2 minutes. In one of these experiments it lasted 4 minutes. The latent period lengthened with the temperature of the bath: 45 seconds at 0° and 4 minutes at 3°.

The attack was also induced at 0° in North American frogs, but not in a steady and constant manner, if any other method was employed. The vertebral column containing the cord was first subjected to the action of an elevated temperature (28 and 36°C.) for 2 to 5 minutes. Then the cord was plunged into a bath of Ringer solution at 0°. Under these conditions, a characteristic attack was observed in many cases; it was not strong, being confined in general to the clonic phase, with the latent period relatively short. In some cases, the attack itself was not produced, but the muscles showed clonic contractions strong enough to persist for 2 or 3 minutes. In a certain number of cases there was no reaction.

5. *Epileptiform attacks produced by chemical excitation of cord.* The application of a crystal of sodium chloride on the medulla oblongata of the frog

produced a convulsive attack (9). Recently Ebbecke (10) also was able to induce convulsions by injecting into the cerebral hemispheres a concentrated solution of NaCl. However, no one has been able to obtain convulsive reactions when the irritant substance acted directly on the cord (2, 9). Moussatché and Vianna Dias (11) have shown that the direct application on the cord of a 20 per cent solution of NaCl provoked a strong convulsive attack in the Brazilian frog. In the North American frogs examined at Yale concentrated solutions of NaCl put in contact with the cord have constantly given rise to an attack resembling perfectly those observed in Rio.

6. *Experiments on Rana catesbyana.* The specimens studied were of middle size, with weights varying from 300–500 g. The muscles of the back were elevated in a manner to reduce as much as possible the surface to be chilled. In no case did chilling at 0° induce an attack or any other reaction whatsoever. In two cases, applications of ether were tried, but without effect. With ethyl chloride a strong attack resulted, both clonic and tonic. One of the frogs treated by caffeine, had no attack at 1°C; there were at most some trembling and clonic contractions. The same frog, after a ten-minute exposure to cold had a slight reflex attack.

## Discussion

Epileptiform attacks induced by rapid chilling of the cord appear in *Rana pipiens* in North America in a manner quite different from those observed in *Leptodactylus ocellatus* in South America. They resemble rather the reactions observed earlier in European frogs. Singly, the chemical excitations give reactions which are the same in *R. pipiens* and *Leptodactylus*, and which are different from those of European frogs.

All these observations raise the question whether the proven differences are due to the fact that there are species differences, or to the action of climate and other conditions of life.

The best means of studying this question is to acclimatize one species of frogs of one country to a different climate in another. At Rio de Janeiro *Rana catesbyana* have been raised for several years being imported from North America. In our laboratory it has been established that these frogs, born in Brazil, did not have attacks at 0° or below. It was necessary to chill the cord below 0° by ethyl chloride, for example, in order to produce the attack. Experiments were repeated every year to establish whether a modification of reactions could be found with the passing of time.

On a recent visit to Cuba it was possible to do some experiments with specimens of *Rana catesbyana*. From the information obtained, this species, also imported from North America, had been in Cuba for about 20 years, a much greater time than that which had elapsed since we had started to raise them in Brazil. This made it possible to verify whether such a prolonged adaptation as this to a warm climate produced perceptible modification of the characteristics of the attack.

In the experiments carried out at Havana, the muscles of the back were

elevated, as in those performed at Yale. The Ringer bath technique, at a temperature varying between 0.3 to 1° was employed. In one case, there was no attack properly speaking, but the clonic contractions were fairly strong and prolonged. In the other cases, clearcut attacks, although feeble, were produced. Naturally the application of ethyl chloride produced much stronger attacks. The attacks observed were similar to those which were found when the cord was subjected to temperatures approaching the upper limit. The temperature of 1 or 1.5° was then, in a similar manner, the actual upper limit of the attacks in *Rana catesbyana* in Cuba. If one compares these results obtained at Yale, where it was established that the attack was not produced in frogs, either at 0° or by application of ether or even by preliminary sensitivation by caffeine, it may be concluded that there is a progressive modification, a slight adaptation of this sort of reactions of the central nervous system to conditions of climate. The fact that *Rana catesbyana* of Brazil did not have attacks at 0°, demonstrates that to induce this alteration needs several years.

## Conclusions

1. In North American frogs, *Rana pipiens*, an epileptiform attack by quick chilling of the isolated spinal cord was not produced at temperatures below 0°C., on the contrary they have been observed in South American frogs (*Leptodactylus ocellatus*).

2. However, the attack can be obtained at 0°C. or a little below if the vertebral canal is opened and the cord directly exposed to the action of cold. In these conditions, the phenomenon, although frequent, was not constant.

3. The attack could be induced by cooling the cord below 0°C., by the application of ether, ethyl chloride, dry ice or by utilizing freezing baths of liquids with low freezing points.

4. The characteristics of the attack depended on the method employed. When the attack was complete, it was prolonged and presented clonic and tonic phases. The feeble attacks were exclusively clonic.

5. The preliminary injection of caffeine in the frog made attacks possible up to temperatures of 3.5°.

6. In many cases, the preliminary warming of the cord for several minutes at a temperature from 28 to 36° inclusive, made it possible to induce an attack at 0° or at least a little below 0°.

7. The direct application of a concentrated solution of sodium chloride on the spinal cord produced in the North American frogs a convulsive attack perfectly resembling that observed under the same conditions in the Brazilian frogs.

8. At 0° or below, *Rana catesbyana* in North America does not on chilling of the spinal cord have an attack.

9. This compared with the fact that frogs of the same species adapted to a warm climate (Cuba) for more than 20 years frequently with attacks at 1° below zero demonstrate that this convulsive reaction of the nervous sys-

tem changes slowly and progressively under the action of the surrounding temperature.

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# EFFECT OF HYPOTHALAMIC LESIONS ON ELECTRICAL ACTIVITY OF CEREBRAL CORTEX

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THERE HAS recently been growing evidence of the functional interrelation between the cerebral cortex and the lower subcortical levels. It seems that the cortical activity is sustained and influenced not only by the afferent impulses from the different sensory organs, but also by the impulses from lower subcortical levels (thalamus, diencephalon, etc.), that play a part in the maintenance and modification of the cortical activity. Exploration of the electrical activity of the nervous tissue has been used in the study of the functional interrelation between the cortex and the lower levels (6, 7, 9). We here report some experiments on the influence of hypothalamic lesions upon the electrical activity of the cerebral cortex.

## METHODS

The experiments were acute and carried out in 20 cats under Nembutal anaesthesia. The electrical activity was recorded from one hemisphere by silver wire electrodes inserted through small holes in the skull until in contact with the dura. The recording was bipolar with a distance of about 1 cm. between each pair of electrodes. Usually three pairs of such electrodes were used. The records were taken with a six-channel ink writing electroencephalograph (Adams and Bradley model). The hemisphere opposite to the one on which the records were taken was exposed by removal of the bone and through this side the different lesions were made. At the end of the experiment the brain was removed and kept in formalin for its macroscopical study.

## RESULTS

In the cat under Nembutal anaesthesia we have observed a spontaneous electrical activity made by waves with a frequency between 5 and 10 per sec. and a voltage of about 40 to 200$\mu$V. This spontaneous activity often appears in discharges of waves with a duration of several seconds (2 to 5) and repeats at intervals of 2 to 10 sec. Sometimes the spontaneous activity is more continuous. This type of spontaneous activity under barbituric anaesthesia conforms to the pattern found by different authors (2, 8, 14).

The lesion of the hypothalamic and basal portion of the brain completely abolishes the spontaneous activity of the cerebral cortex (Fig. 1 and 2), for the duration of the experiment (one or more hours). It is important to emphasize that this effect of hypothalamic lesions on the cortical activity takes place with the complete integrity of the thalamus and the rest of the brain*.

The interrelation between the hypothalamus and the cerebral cortex seems to be through the thalamus. The anatomical evidence (5) shows two main pathways from the hypothalamus to the thalamus. One is from the mammillary bodies to the anterior nucleus of the thalamus. The other path-

* This abolition of cortical activity does not seem to be secondary to changes in the circulation or blood supply of the cortex, because injection of stimulating drugs (strychnine, metrazol) brings back temporal and epileptiform discharges of the cortex.

way, which is perhaps more important, is between the posterior hypothalamic region and the medial nucleus of the thalamus, through the periventricular system of fibers. It is known that lesions of the thalamus or the thalamo-cortical radiations also abolish the activity of the cortex (1, 2, 3, 9, 11, 7). We have also observed the disappearance of the spontaneous activity of the cerebral cortex by thalamic lesions or by section of the thalamo-cortical connections. Dempsey and Morison (6) have observed that even a small portion of cortex (1 cm. sq.), isolated by careful removal of all the cortex of the hemisphere and keeping its thalamic connections, can maintain its spontaneous bursts of activity. Besides, there is also evidence that stimulation of

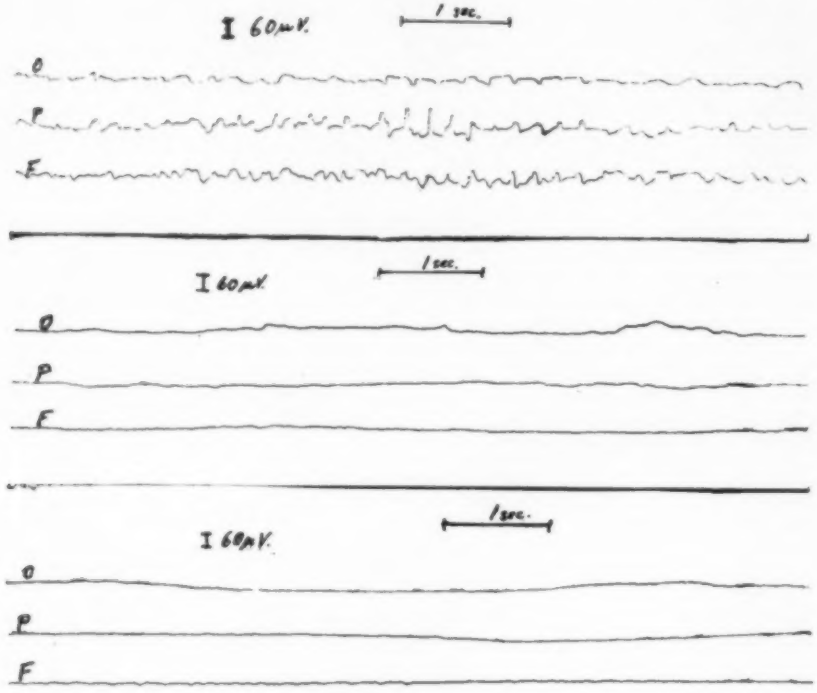


FIG. 1. Spontaneous cortical activity before, immediately after and 50 min. after lesion of the hypothalamus. O occipital, P parietal and F frontal leads.

the medial nucleus of the thalamus gives rise to widespread cortical responses similar to the bursts of activity of the spontaneous electrocorticogram (6, 7).

It can be assumed, therefore, that the hypothalamus may influence the cerebral cortex through its thalamic connections and perhaps by way of the medial thalamic region. The driving influence of the hypothalamus upon the cortex has been shown by experiments with hypothalamic stimulation (10). Cortical responses also occur after stimulation of the subthalamic regions (13).

There is also clinical evidence to support abnormal slow waves in the cerebral cortex in cases of tumors involving the diencephalon and structures around the third ventricle (4, 15). It is difficult to find an explanation for these slow dicharges and Adrian has suggested, in the case of Cairns, Oldfield, Pennybacker and Whitteridge (4), that the pressure on the thalamus by the tumor may have sent up abnormal volleys of impulses which might be responsible for the appearance of large waves on the cortex.

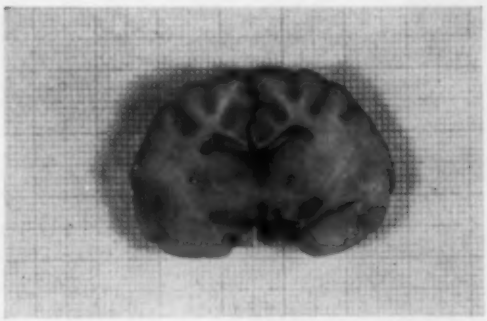

FIG. 2. Section of the brain of the cat used in the experiment of Fig. 1 showing the lesion of the hypothalamic and basal region of the brain.

The lower levels below the hypothalamic and basal region of the brain do not seem to have such an intense and prominent influence on the cortical rhythms. The cortical activity persists, at least for some time, in the cat (2) and monkey (12), after completely sectioning the midbrain. In our experiments we have also confirmed the persistence of cortical rhythms, although diminished, after sections through the midbrain, even at the rostral level of the superior colliculi bodies.

## SUMMARY

A lesion limited to the hypothalamus and basal region of the brain abolishes the spontaneous electrical activity of the cerebral cortex.

After lesions of the thalamus or its thalamo-cortical pathways, the cortical activity also disappears.

On the basis of different kinds of evidence it is suggested that the hypothalamus may influence cortical activity through its thalamic connections.

Section through the midbrain does not entirely abolish cortical activity.

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# ELECTRICAL POLARIZATION OF PACEMAKER NEURONS*

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## INTRODUCTION

INDICATIONS are now numerous that the frequency of firing of the nerve cell depends on the DC electric field in which it lies ("electrotonic current," "somatic potential," "standing or steady state potential"). Such DC fields are known to exist in nerve cell masses (19, 15) and evidence is accumulating that imposed constant currents will exert profound effects on the nerve cell. Direct evidence that imposed DC alters many properties of the nerve fiber has been obtained (14, 29, 13). Gerard (9) has reviewed the evidence that "cell discharges are related to cell potentials" (see also 14). A number of arguments have been brought forward to support the view that the cell body fires according to the over-all DC field resulting from the number, time relations, and spatial distribution over its soma of the axon action currents converging on it from many sources (10, 11).

A direct demonstration of a change in the frequency of firing of nerve cells by altering the intensity of an imposed DC field has been made by Barron and Matthews (5) in spinal motor neurons of the frog and cat. The experiments of Auger and Fessard (3, 4), and Bradway and Moore (7) may at least include this effect. Changes in the vertebrate electroencephalogram during and following the passage of direct current through the brain, have been reported (8, 19). Changes in frequency were not present or not prominent and in any case the nature of these waves and their relation to nerve cell firing is too little understood to allow interpretation of them in terms of individual nerve cell behavior or resulting axonal impulses.

A definite effect on frequency of heart beat has been obtained in *Cladocera* (24), molluscs (2, 20, 22), and vertebrates (31, 23, 25, 30, 21, 16). But the effects are probably not exerted on nerve cells in any of these cases. They differ in character from those here described since they are at least partially adapting.

The ganglion of the neurogenic heart of *Limulus* seemed to offer an exceptionally favorable opportunity to test the question on nerve cells with an intrinsic automaticity. The preparation is favorable for handling, dissecting and electrical recording. The purpose of this paper is to answer the question whether an imposed constant current acting directly on the ganglion will

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alter the frequency of discharge of the pacemaker neurons of the cardiac ganglion of *Limulus* to affect the over-all heart rate. The attempt is not made here to analyze the effect on the frequency of discharge of single cells within each heart beat.

## MATERIALS AND METHODS

About three dozen adult specimens of *Limulus polyphemus*, mostly females, collected in Long Island Sound between March and June, have been studied in detail, by continuous electrical recording over periods of several hours. The specimens were kept in air at room temperatures for periods up to a week before use. The heart was exposed by removing the exoskeleton over it, carefully cutting the attachments of the pericardium. In most cases the organ was then removed from the body carefully and cleaned of pericardium and gonadal tissue. Lying on a horizontal glass plate, in air, at room temperature, the heart beat was remarkably steady in amplitude and frequency, for many hours. Usually no sea water, artificial physiologic solution or natural blood was used to bathe the heart. Little trouble was encountered from drying. It was preferred not to add complications of mechanical stimulation or change of chemical environment, and complete immersion was undesirable for electrical reasons. In some cases, however, it was necessary for special reasons to have a circumambient fluid or to wash the preparation. Both sea water and serum drained off after coagulation of the blood have been used. No differences between them have been noted.

The frequency and amplitude varied considerably between preparations. No correlation with condition of the animal, character of the dissection or other obvious factors was apparent. But both frequency and amplitude could be modified by conditions such as temperature, pH, state of distension, etc.

Certain experiments called for partial or complete removal of the ganglion. This was done by lifting the ganglion at one end and cutting beneath it with fine scissors carefully avoiding inclusion of muscle. If the dissection was carried far enough so that no pacemaking neurons were left attached to the myocardium (as tested by complete cessation of beat on cutting through the connection between ganglion and myocardium) not all of the heart but only the half or third of it nearest the undissected end continued to beat. If the separation was not carried so far the whole myocardium continued to beat but some portion of the ganglion capable of becoming a pacemaker was still attached to the muscle.

Polarization was accomplished by passing currents through the tissue by way of Ag-AgCl-sea water electrodes of convenient form. Leads came from the movable points of two parallel potentiometers across a series of dry cells. A galvanometer with a range switch permitting accurate readings of current flow from a few microamperes to milliamperes, was in series with one lead. A polarity reversing switch was included when desired. The current was introduced, increased or turned off by rotation of one of the stepless carbon potentiometers, although it was found that a sudden make or break was just as satisfactory and did not introduce special complications due to stimulation.

The electrical activity of the heart was recorded chiefly with moving coil, siphon pen oscillographs using Grass amplifiers (condenser-coupled) but many records were also made with the DC amplifier of Goodwin (12) and with moving coil, loop (Westinghouse) or cathode ray oscillographs. Three channels of recording were usually used simultaneously. Ag-AgCl-sea water electrodes in glass pipettes with cotton wicks were employed, several pairs usually being applied to the heart, each electrode mounted in an individual polystyrene holder.

Mechanograms were taken occasionally. This was done either by a thread which transmitted the transverse contraction of the heart to a hinged shutter intercepting a light beam falling on a photo-cell, or by inflating the heart with air and illuminating the photo-cell with a mirror mounted on a sensitive tambour in communication with the heart. Thus the mechanogram could be recorded by one of the amplifier-oscillograph channels on the same record as the electrical activity.

## RESULTS

The character of the electrogram of the intact heart of *Limulus* is seen in Fig. 1 and 2. It is of complex form; most of the deflections are not dupli-

cated exactly in successive beats, but the initial and largest waves are constant in form (over several hours, in a given preparation, from any one pair of electrodes) and the duration of the burst of electrical activity as well as the interval between beats is constant within rather narrow limits. The electrogram of the intact heart is probably to be considered as purely an electromyogram. The electrogram of the ganglionic burst (Fig. 1 and 5) displays

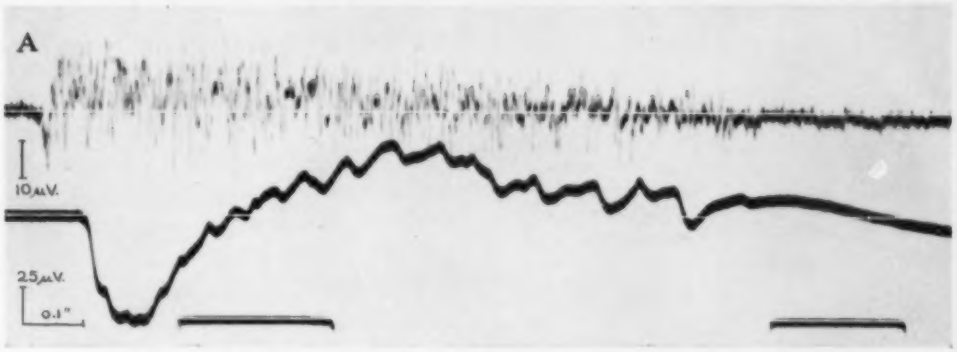


FIG. 1. Electrograms simultaneously taken from (upper record) ganglion and (lower record) intact surface of the heart in a preparation similar to Fig. 4. Grass amplifiers and Westinghouse loop oscillograph. Filters for upper record passing from slow (time constant −0.3″) to fast components. Filters for lower record passing from about 2 to 50 per sec. This upper limit is not responsible for the smooth line. Its character is not changed by switching to high pass filters. The fast activity of the ganglion does not show presumably because its amplitude is too small by reason of tissue shunting. Note the "DC" component in the upper record—a rapid rise and slow fall of the average potential. The slow component of the lower record has been attenuated to keep the excursions on scale.

If a mechanogram were shown its deflection would begin about 0.5 sec. after the initial deflection of the lower record and rise smoothly to fall after the electrogram has become quiet. A common observation, frequently more pronounced than in this record, is that the first indication of a heart beat is not a spike in the electroneurogram but a slow shift of the DC base line (cf. 6).

the same general constancies although the form and frequency spectrum of the component deflections are of an entirely different order (1, 17, 18, 26, 27, 28).

*Effects of polarization of intact heart.* In general, the result of passing small DC currents through the heart is an immediate, sustained, reversible change in frequency and character of the beat and the effect is graded with graded values of current passed.

The effect on frequency is the most obvious. Figure 2 shows a sample record and Fig. 3, a plot of the heart rate against current passed for a typical experiment. It will be seen that the effect, in both directions of current passage, is an increase in frequency. This result is uniform throughout the experiments. No consistent and reproducible, indeed no considerable reduction in frequency, has been obtained with any placement of the electrodes. There may be slight but consistent differences between the effects of the

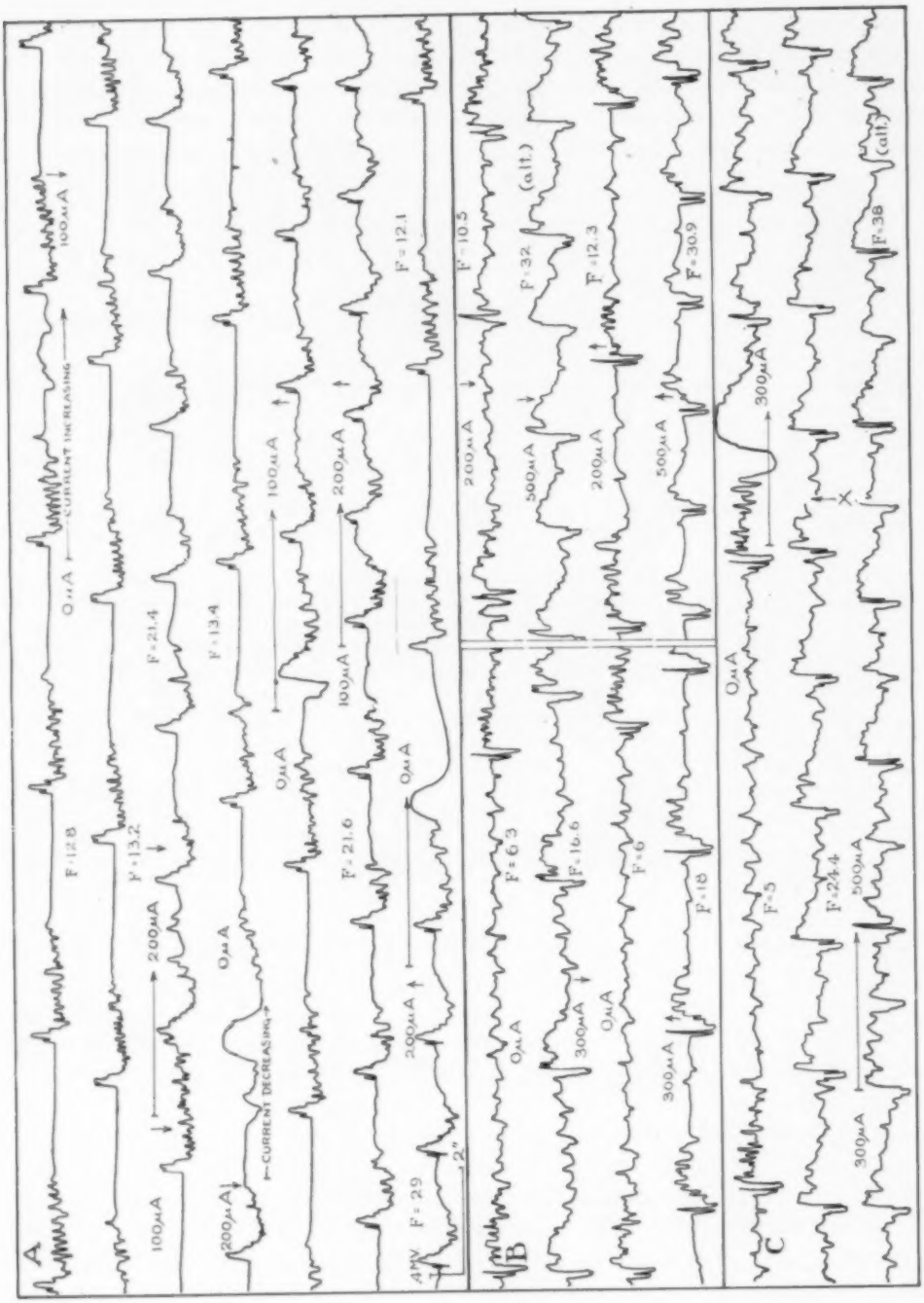


FIG. 2. For legend see opposite page.

two directions of current passage in a given preparation and for a given pair of electrodes. But the character and direction of the differences were not consistent between preparations. Experiments reported in a later paragraph may provide an explanation of this inconstancy.

The heart in Fig. 3 was accelerated more than six-fold by moderate currents. This represents an increase almost exactly proportional to current for the "ascending" polarization, and more than proportional to current for the "descending" polarization. But the acceleration possible and the degree of proportionality vary among preparations, chiefly due to (i) the "resting" or zero-current frequency that happens to obtain in that specimen, (ii) the maximum frequency of which it is capable under the conditions of the experiment, (iii) any fatigue or gradual drop in "resting" frequency that is occurring, as well as (iv) its actual sensitivity to the imposed field. In these experiments the zero-current frequency has varied commonly between 5 and 18 beats per min., the maximum rate of beat of the intact (polarized) heart from 40 to 50, approximately. The constancy of the interval between beats was commonly better than 95 per cent. That of the number of beats per minute is harder to ex-

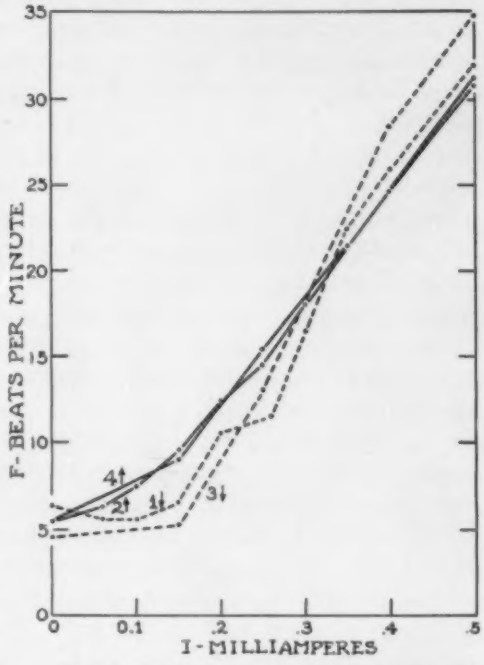


FIG. 3. Relation of heart rate to current imposed in a typical experiment. Intact excised heart polarized between fifth and seventh segments through cotton wicks resting on surface of heart. Points on curve obtained in succession from lower to higher currents and curves in the order numbered.

FIG. 2 (A). Electrogram of intact, excised heart under polarization, showing change in frequency of beat and in wave form of initial deflections. Note shorter duration of each beat as well as shorter interval between beats, during polarization. Currents turned on and off gradually (long arrows). Pickup from second segment of heart and sea water bath. Imposed current passed from fourth to sixth segment or vice versa; upward pointing arrows indicate "ascending" current (sixth segment anodal), downward pointing arrows "descending" current (sixth segment cathodal). Grass amplifier and ink writer. "F" = frequency of heart beat in cycles per minute calculated from measuring record.

(B) Another preparation; samples of a continuous record, similarly obtained. Note effect of polarization on wave form and different effect of the same current passed in opposite directions. Alternation of two wave forms at 500$\mu$A, "descending."

(C) Same heart as B. Polarization experiment some moments later showing abrupt character of change from one frequency and wave form to another. Continuous record; about 20 sec. omitted at "x". Two alternating wave forms at 500$\mu$A.

FIG. 2. For legend see opposite page.

press, for the same heart may vary less than 1 per cent over many minutes or it may change as much as 10 per cent (rarely) in succeeding minutes. Ordinarily the number of beats in 20 sec. have been counted and computed as beats per minute. The "resting" rate usually varies in the course of an experiment of several hours, including some relatively drastic polarization, by not more than 10–15 per cent. The frequency at a given current value is at least as constant as this and is ordinarily within the accuracy of setting the potentiometer.

Absolute values of current mean little because of the difficulty of defining the current density in the tissues. The points of greatest current density are probably the points of contact of the electrodes. The electrodes in these experiments were wicks whose diameter at the point of contact was two to three millimeters. Threshold currents were usually between 60 and 150$\mu$A. A frequency increase somewhere near proportionality has in every case been realized between 150 and 500$\mu$A. At some point between 500 and 1500$\mu$A the increase is checked, the beat becomes small, irregular and difficult to count and may stop. But at any point in the experiment, even after apparent stoppage of the heart, interruption of the imposed current is followed immediately (*i.e.*, within the period of one beat) by resumption of the normal beat. There is often a slight deficit or excess over the initial "resting" rate and a gradual recovery over a period of minutes. (The lack of perfect reversibility in many preparations may be attributed in some cases to effects of the current—either fatigue or stimulation may occur, but in others it seems probable that a slow progressive change was taking place independent of the polarization. These effects were never great enough to overshadow those of imposed current and were often absent altogether.) With moderate currents the abrupt, maintained character of the effect and of its cessation is marked and the virtual absence of slow adaptation or stimulation after both "on" and "off" is characteristic.

Simultaneous with the change in heart rate during polarization is a change in the character of the beat. This may not be obvious in the mechanogram but is clearly shown in the electrogram of the intact heart. Moderate degrees of polarization result in slight or considerable changes in relative size of the initial and large waves of the electrocardiogram. There is usually also a reduced duration of electrical activity associated with each beat. Often a polarized heart will exhibit a wave form radically different from its "resting" wave form, none of the individual waves of the one form being recognizable in the other until the intermediate steps are studied. The wave form of the polarized heart may be quite different in the two opposite directions of current passage, though the frequency may be the same. With relatively high currents the electrocardiogram becomes smaller in amplitude, shorter in duration, greatly changed in form and, moreover, at a certain point, irregular. The irregularity may be only in form, so that successive beats are not alike, or in frequency, or both. Eventually as the current is increased, all these changes accumulate to render it impossible to count the

beats, often paralyzing the heart. The mechanogram may be completely inactive and no contractions whatever visible upon close macroscopic examination of the heart while distinct activity in the electrocardiogram is still present. The heart is in diastole. At still higher currents when the electrocardiogram has ceased activity, its DC level is that of rest, not a smoothed out tetanic series of spikes. With somewhat lower currents while the mechanogram still follows the electrocardiogram it may lose amplitude before the electrical record; the contractions may become local, various parts of the heart not being synchronized; the mechanogram often skips beats and has been recorded for a long period responding to every third, fourth or fifth beat of the electrocardiogram. (It should be recalled that the electrocardiogram under discussion throughout this section is supposedly an electromyogram.) Commonly, at a certain high or even moderate current value, the succession of heart beats suddenly exhibits an alternation between two regularly recurring consistent wave forms. One of these is usually recognizable as the previous sole wave form, the other being entirely new. Often the frequency curve shows no break at this point but continues to rise steadily (see Fig. 2). Every stage in this series is immediately reversible, the original wave form being perfectly restored on cessation of polarization, even though many minutes have elapsed since that form was last exhibited. Moderate degrees of polarization can be maintained for long periods (30 min.) with perfect reversibility of wave form and only slight loss or gain in frequency.

All the effects just described were obtained by polarizing longitudinally from one electrode on the mid-dorsum of the heart to another more anteriorly or posteriorly. The attempt was made to discover the influence of the location of the electrodes. It was found that moving either or both electrodes forward or back along the mid-dorsal line had only a slight effect on threshold, within the segments of the heart occupied by the ganglion. Moving both electrodes away from the ganglion, however, for example one into the first segment, the other far laterally in any segment, resulted in a large change in threshold, raising it so that even currents of 500–1000$\mu$A only slightly or not at all increased the heart rate. The same was true if both electrodes were dipped into sea water pools in contact with the heart, restricted such that the current had to pass through the heart, whether the electrodes were on opposite sides of the heart, right and left, or anterior and posterior to its ends or in any other position. The suggestion arose therefore that the effect of imposed currents as applied earlier was a local one dependent on the points on the heart touched by the electrodes, these points having the highest current density, and various regions of the heart being differentially effected by the same current density. If this is correct, several interesting implications would seem to follow. Since the heart tissue and the film of salt water on its surface are good conductors, the current density will be highest at the point of contact of the electrode and will rapidly fall off with distance therefrom, the current path widening in a cone-like fashion. This might mean that one has little control over the orientation of the effective portion

of the field, that the position of the second electrode would make little difference, providing it was situated where the high current density under it did not traverse a tissue of high sensitivity. That is "ascending," "descending," transverse or other axes between electrodes might be expected to have only slightly different effects. Further, it might be expected that much greater differences would be realized by changing the electrode over the sensitive tissue from an anode to a cathode, than by keeping it, for example, a cathode and moving the anode around it 360°.

To test these matters the heart was polarized with one electrode in a sea water pool broadly in contact with the heart, the other ("stigmatic") electrode touching the tissue. It became immediately apparent that a great difference existed between passing current from the stigmatic to the diffuse electrode and passing it in the opposite direction. The difference seemed to be one of effectiveness or threshold for the same modifications of heart activity. The cathode was the more effective stigmatic electrode. At a given moderate current level the stigmatic cathode commonly exerted eight to twenty times as great an effect in percentage increase in frequency as the stigmatic anode.

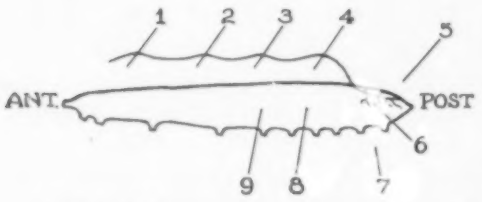


FIG. 4. Diagram of the preparation with ganglion partially lifted off. The excised heart is carefully dissected to separate the ganglion, lifting it on wick electrodes, from either anterior or posterior end, leaving the other end attached to the heart. Ganglionic discharges continue to excite all of the myocardium reached by the nerves intact. With numerous electrodes in place (numbers correspond to text references), electrograms of the ganglion alone, the myocardium, the intact portion near one end and of regions within each of these may be recorded. Polarization may be imposed through any pair and the effects picked up at several points. The heart lies horizontally on a glass plate in a shallow pool of sea water or serum, not submerged. Electrodes 5 and 7 are in this fluid.

The lack of effect when polarizing through the lateral or anterior muscle or its high threshold relative to the region of the ganglion was clearly demonstrated. Slight differences were found between different levels of the ganglion. It is probably this kind of difference which is shown by Fig. 3—reversing the current is actually shifting the effective electrode (cathode) to a different part of the ganglion. If the direction of current passage is reversed not by reversing the polarity of fixed electrodes but by leaving the cathode in one place and moving the anode now anterior, now posterior, to it —or to the right or the left, close or far away, on the heart or in the sea water pool, no difference is found at moderate currents. The position of the anode is uncritical, that of the cathode is very critical as regards on or off the ganglion, and moderately critical as to the level of the ganglion.

*Polarization of ganglion and muscle separately.* A number of preparations of partial or complete separation of the ganglion from the myocardium were polarized in various ways. It was hoped to confirm the notion that the ganglion was the site of action of the imposed current and it was supposed that

the dimensions of the ganglion—about those of the wick electrodes—would, by confining the current path, provide a more nearly constant current density through some length of the ganglion and perhaps reveal differences between "ascending" and "descending" currents distinct from the effect of the position of cathode or anode.

The first question was easily answered when polarization of the ganglion-free or near ganglion-free muscle produced no effect on heart rate or wave form up to currents of several milliamperes, whereas 20$\mu$A through the ganglion along, or only a small portion of it, sufficed to speed up the beat of that same muscle by fifty per cent or more. (A diagram of the preparation discussed in this section is given in Fig. 4.) Cutting across the last nervous connection between ganglion and myocardium, but leaving the cut nerve in contact with the muscle—thus interrupting physiological but not electrical connection—obliterated the effects on the muscle. But leading off from the ganglion, its discharges could be recorded and the polarization effect seen to be still present in them.

The problem of polarity was more difficult. Under the conditions of the experiments it was possible to show that neither cathode nor anode was consistently more effective and that direction of current flow did not have a consistent effect. Polarization was effected through various combinations of the electrode positions shown in Fig. 4 and the electrogram of the ganglion or/and muscle led off from other pairs not lying in the current path. Threshold currents were between 5 and 12$\mu$A, marked acceleration occurring at 20$\mu$A and many preparations exhibiting the signs of high current described above, at 30 or 40$\mu$A. All the phenomena described for the electrogram of the intact heart were demonstrable in the high frequency ganglionic bursts as well. Consistency of major features of wave form, alternation of two definite wave forms at high currents, shorter bursts at higher frequencies, and the abrupt, reversible character of the effect are some of these (Fig. 5).

When the polarizing electrodes were any two of electrodes 1–4 (Fig. 4), thus both on the ganglion, there was, in some cases, a difference in the frequency at a given current level when the polarity was reversed. This, of course, may be due to the different position of a more effective electrode as in the intact heart or to the direction of current flow through some critical region of the ganglion. But in most cases there was no appreciable difference. When one electrode was in the circumambient sea water (5, Fig. 4) and the other was on the ganglion (1, 2, 3 or 4, Fig, 4) the small currents necessary to effect the lifted portion of the ganglion were far below the threshold for any ganglionic tissue still on the heart, or for the myocardium. The results of polarizing in this way were distributed among all the possible results. In some cases there was a greater effect when the electrode on the ganglion was the anode, in some cases when it was the cathode, in the rest there was no significant difference in effect when the current was reversed. As with the intact heart, such differences as were found were in threshold and degree of acceleration; a reversal of effect, *i.e.*, deceleration, was never encountered.

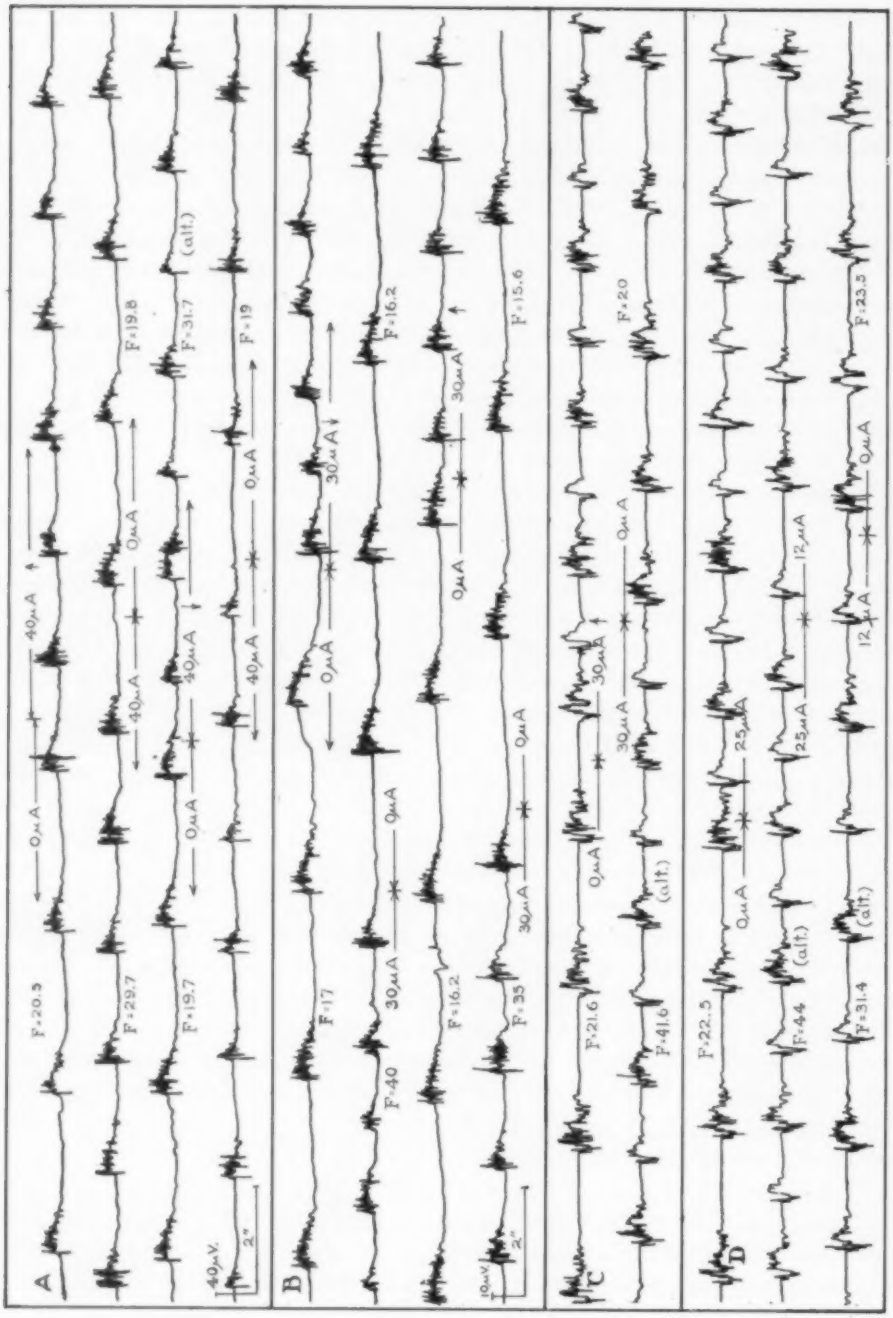


FIG. 5. For legend see opposite page.

## Discussion

It seems probable from the evidence presented that the effect of an imposed direct current is a direct result of the electrical field itself. The abrupt character of the change in heart activity at "on" and "off" as well as the steady (non-adapting and non-accumulating), character of the effect during current passage argue against an indirect result of such factors as temperature rise, or products of electrolysis. Whatever the mechanism, it is one which comes to equilibrium within a fraction of a second. Furthermore it may be characterized as increasing up to a certain point directly with the current, apparently a function of current density, *i.e.*, field intensity. It can act locally, altering the activity of the whole heart by acting on a few cells. And it apparently does not alter the intrinsic electrical field or any other property that cannot return to its original state in a fraction of a second.

The site of action seems to be the ganglion and apparently may be some critical point in the ganglion. Any possible changes in peripheral nerve or muscle do not seem to enter into the effect as measured in these experiments.

There remain the questions why no consistent relation to gross anatomical orientation is evident and why the effect is obtainable in only one direction, *i.e.*, increased frequency. The evidence from the present experiments does not afford an answer but it may be that there are consistent relations with the microscopic anatomy of the ganglion. The histology is insufficiently known but it seems more than probable that the nerve cells are oriented in all directions. If this is so and the actual effect of the DC field is determined by its orientation in relation to the polarity of individual cells then one might expect no consistent relation to gross anatomy. Moreover, since it is evident that the pacemaker of the entire heart may be quite localized—perhaps one cell—and that it may shift, that is, many potential

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Fig. 5. Electrograms of the ganglion of a preparation similar to Fig. 4. Grass amplifier and ink writer. The fast components of each burst of activity are not recorded faithfully by the ink writer (see Fig. 1) but the time relations and slow components are, down to about 2 per sec. Electrode numbers correspond to those of Fig. 4.

(A) Polarizing between electrodes 3 and 4, pickup 1 to 2. Current made and broken abruptly. First two lines consecutive, 80 sec. omitted between lines 2 and 3, 70 sec. between lines 3 and 4. Note abrupt maintained change in frequency and duration of bursts at make and break of currents.

(B) Same preparation, polarizing between electrodes 4 and 5 so that a short segment of ganglion becomes alternately anodal and cathodal with respect to a diffuse electrode, as current is reversed. First two lines consecutive, last two lines consecutive, 2′, 40″ omitted between lines 2 and 3.

(C) Same preparation, polarizing between electrodes 1 and 2, pickup 4 to 5, showing alternation of two types of burst. Two lines consecutive. Calibration as in B.

(D) Same as C. Three lines consecutive. Alternation at 25$\mu$A appears to double the frequency simply by adding a new (simple, shorter) burst midway between existing ones, but at 12$\mu$A, overall frequency still higher than normal, the bursts are still evenly spaced, thus the "original," or any one, wave form is actually slower than normal. The frequency determining mechanisms must control the overall frequency rather than simply adding extra beats or starting a new independent pacemaker.

pacemakers exist in different parts of the ganglion, presumably with different anatomical orientations, it is quite conceivable that any decelerating effect of a given DC field would be exerted only on certain cells and would be masked by the accelerating effect of the same field on other cells, which would then take over the pacemaking function. Thus a new pattern of cells would initiate the heart beat and this may account for the difference in wave form of the electrocardiogram during polarization and for the difference in wave form at the same frequency with opposite current polarities.

## Summary

1. The isolated heart and cardiac ganglion of *Limulus* has been used as a test of the hypothesis that the frequency of firing of nerve cell bodies depends upon the DC electrical field in which they lie.

2. It is shown that a direct current passed through the intact heart results in an abrupt, sustained, reversible increase in frequency of heart beat and in changes in wave form of the electrogram.

3. The effect apparently is exerted locally, at the point of contact of the electrodes and the tissue, where the current density is highest. It is usually confined to the cathode in preparations of the intact heart. The anode and the gross orientation of the field appear to be uncritical. In preparations of the isolated or partially isolated ganglion the cathode was not consistently more effective.

4. The effect has been obtained only in the direction of an increase in the normal frequency. A possible explanation of this is offered.

5. It is concluded from the abrupt non-adapting, non-accumulating and promptly reversible character of the effect, that it is a direct effect of the DC field.

6. The site of action is apparently the ganglion, more specifically the region of the pace-making cell bodies.

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# SPECIFIC EXCITABILITY OF THE ENDPLATE REGION IN NORMAL AND DENERVATED MUSCLE

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## INTRODUCTION

SPECIFIC properties of the endplate region have been described by Langley (28) who showed that small concentrations of nicotine excited muscles around the nerve entry, while much higher concentrations were required for a similar effect on other regions. The "receptive substance" was regarded as possessing different properties from nerve and muscle. Lucas (30) found a distinct electric excitability of the neural region in the frog's sartorius—the $\beta$ excitability—but this could not be shown by other investigators (33).

In a series of experiments in normal and chronically denervated muscle, drugs were applied to the 'nervous' and nerve-free regions of the whole sartorius and to the endplate and other parts of single nerve-muscle fibre preparations (25), while the potential changes at the place of application were recorded. It seemed of special interest to investigate the effects of acetylcholine and potassium besides other substances as nicotine and caffeine.

Some authors (11, 12) found longitudinal potential gradients in resting muscle fibres and also potentials localized at the end-plate region. Special properties of the endplate itself could be found in the present investigation but with the technique employed, no appreciable longitudinal potential gradients were detected in uninjured resting muscle fibres, either localized around the endplate regions or on other parts of the muscle.

## METHOD

Frog's sartorius or isolated nerve-muscle fibre preparations from the M. adductor longus of the Australian *Hyla aurea* were used. The potential changes were recorded with a resistance-capacity coupled amplifier, the response usually falling to one half in about 2.0 sec. occasionally 5.0 sec. when large coupling condensors were used. Although the potential changes after local application of drugs (Section II A, 1) lasted for several seconds only those taking place during 0.2–0.5 sec. after application are discussed here.

*Technique of drug application.* (i) The single nerve-muscle fibre preparation was lifted into paraffin while two electrodes made contact with it. One electrode was usually on the end of the muscle fibre while the other could be moved to different positions with the help of a micro-adjustment. By means of a small glass hook containing less than $0.3\mu$ l. of solution drugs were applied under direct microscopic observation (Fig. 1). When the recording was done from the region of application, the drugs were put on the tip of one recording electrode in contact with the muscle. The small drop could be held there in position by the glass hook. When platinum electrodes were used, the application of ACh or KCl produced an initial positive potential. Since this also occurred when the muscle fibre was

substituted by a fine thread of cotton soaked in saline, it was clearly a potential originating at the electrode-drug liquid junction. Such potentials and also the electrode polarization could be minimized by the use of silver-silver chloride electrodes making contact over about 1 mm. fibre length by a fine thread of cotton wool soaked in Ringer (27). Sometimes the greater part of the applied drug soaked into the cotton wool wick and a variable dilution came into contact with the muscle fibre. Usually, however, the drop could be applied and kept under the hook while the recording was done. Application of such a drop effectively broadens the contact area of the recording electrode to about 2 mm. Thus the contact is not very well defined and it is not possible to restrict recording accurately to such a very small area as the endplate. Estimation of the actual size of potentials at such small regions can be made by comparison with the sharply localized interface leading (25, 26).

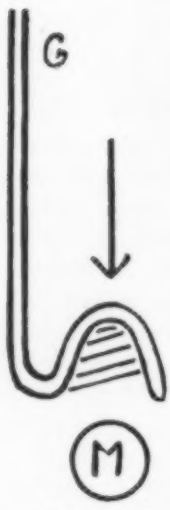


FIG. 1. Diagram illustrating local application of drugs to an isolated muscle fibre M (transverse section). G, glass rod terminating in a fine hook. Shaded area shows droplet of the drug which is brought into contact with the muscle fibre by lowering the glass rod (arrow).

Photographic records were taken on a slow falling-plate camera or by using a thyratron time base.

(ii) When long lasting potential changes after drug application were measured, the following method was employed in isolated nerve-muscle fibres and in the whole sartorius. The test solution was put into a small glass dish, which in its turn was placed in a bath of paraffin oil. The muscle was held by two forceps in a vertical position, while still in paraffin, and was lowered into the dish underneath containing the test solution. Thus the muscle could be immersed to a required degree, while one electrode was kept on the muscle in paraffin, the other being in the drug-saline solution. This latter electrode would record changes caused by the drug action at the saline-paraffin interface. The potential which developed was recorded by non-polarizable silver-silver chloride electrodes (as above) and could be balanced by addition of a constant potential to the recording circuit. When in the balanced condition a shortcircuiting key was placed across the input and then opened, no deflection resulted. A constant potential input of 50 mV showed no appreciable alteration during 30 to 40 min. continuous application into the recording circuit. Hence, electrode polarization would not seriously distort the potential readings.

The above method was also suitable for the investigation of potentials along the normal fibre, especially around the nerve entry. But usually the whole nerve-muscle fibre was lifted into paraffin while one electrode remained at one end of the muscle fibre and the other was moved to different positions. In the early experiments potential changes were found, but these were due to alterations in grid current in the input stage which varied inversely with the preparation resistance, as the interelectrode distance was altered. This was eliminated by the use of a preamplifier of high input impedance. For the denervation experiments the sciatic was cut about 1 cm. above the thigh and the frogs were kept from 2 to 11 weeks at room temperature (16–20°C.) and were fed twice a week by insertion of meat, usually liver, into their mouths. Successful dissection of single muscle fibres was more difficult as injury due to manipulation seemed to occur more frequently and fibres did not recover as readily after treatment with the various drugs.

In the single fibre preparations from denervated muscle the location of the endplates could not be discerned under the microscope. In the normal muscle, however, the endplate free regions can be established with reasonable certainty during the dissection. Usually all the fine nerve branches are well visible under suitable illumination and generally they innervate bundles of muscle fibres over a small circumscribed area (cf. multiple innervation in isolated muscle fibre preparations 27).

Altogether 65 preparations were successfully experimented on during the present investigation; 28 were dissected nerve muscle fibre preparations. Sometimes the latter were used on 2 successive days and were kept over night in Ringer at a temperature of 10–15°C. Acetylcholine B.D.H. was used in all experiments. The different KCl concentrations were obtained by diluting in Ringer KCl solution isoosmotic with it. One per cent pure nicotine and one per cent caffeine stock solutions were employed.

## RESULTS

### I. MEASUREMENTS OF POTENTIAL DIFFERENCES ALONG MUSCLE FIBRES

In 10 single nerve-muscle fibre preparations careful measurements of potential differences were made between various points on the surface of uninjured muscle fibres. The innervation of the muscle fibre was preserved and the endplate was clearly seen. The location of other endings on the same muscle fibre could be established in most preparations (cf. above). The condition of the preparation was checked frequently by recording at different positions the muscle action potentials set up by nerve stimulation. The action potential size varied in different preparations and was generally between 50–100 mV, depending on the shunting effect of tissue rests along the fibre. At the end of the experiments the muscle was usually injured by crushing or by KCl application and 'resting potentials' were recorded. These were smaller than the spikes in single fibre preparations. In all preparations random potential differences of 1–2 mV were observed between different parts on the uninjured muscle fibre. In 3 experiments potential differences as high as 4–5 mV were recorded but in each case these potentials were not related to the known endplate. All these preparations might have had slight injuries, but they continued to contract normally for 1–2 hours after the potential measurements were taken.

In 5 experiments the measurements were repeated about 12 hours after the muscle fibres had been 'killed'; in these the protoplasm was 'broken up' and, as expected, no potentials at all could be detected.

In frog's sartorius the endplates are usually located in foci several millimeters from the nerve entry (24). There, potentials around the endplate region—if present—should be recorded. However, in 20 preparations in which subsequently potentials due to drug application (Section II) were measured, no potential differences around the endplates were found under resting physiological conditions. Thus large potential differences along the surface of muscle fibres especially between the endplate and the surrounding muscle as found by Buchthal and Lindhard and also others (cf. monograph 1939) could not be detected in frog's muscle (but see discussion).

### II. A. NORMAL MUSCLE

(1) *Local application of drugs and recording from nerve muscle fibre preparations.* When ACh is applied to the endplates directly (13) or injected into the circulation (9, 5), it has a powerful stimulating action on muscle fibres. Application to the surface of the sartorius sets up a train of impulses, the threshold concentration being at least 10 times higher at the pelvic end than elsewhere along the muscle (19). The stimulating action of ACh at the endplates was readily confirmed in the isolated nerve muscle fibre preparations, but no impulses at all could be set up at other regions. Even 1000 times the threshold concentration at the endplate failed to produce more than a small local contracture at an endplate-free region. This agrees

well with Buchthal and Lindhard's (13) findings on lizard's motor endplates. Other drugs, as nicotine, KCl and with some exceptions caffeine, which sometimes failed to set up impulses, showed the same selective excitatory action at the endplate region. Figure 2Aa illustrates a train of impulses set up after application of $10^{-6}$ ACh to a motor endplate of an isolated single muscle fibre. An initial negativity of the endplate is observed to lead up to the origin of the first spike, and each successive spike seems to be initiated again by a similar potential which is built up again after the refractory period of the preceding impulse. If the same or even a much higher concentration of ACh is applied to a spot a few mm. from the endplate, practically no potential is recorded from the region of application.

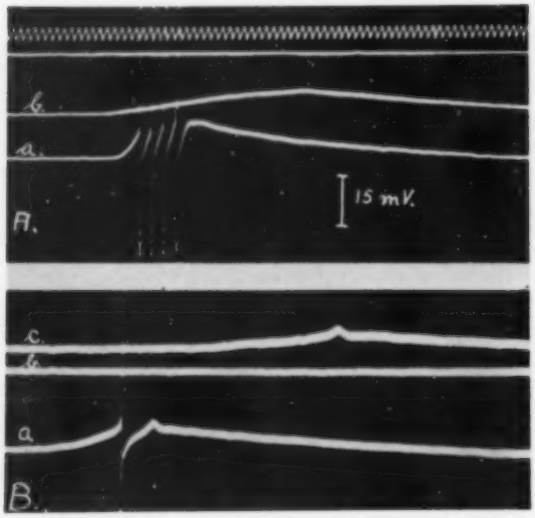


FIG. 2. Local application of acetylcholine to the endplate region of a single nerve-muscle fibre preparation. Recording is done from the point of application. A. a, $10^{-6}$ ACh sets up four propagating muscle impulses. b, a weaker concentration of ACh produces a local negative potential only. B, c. $10^{-6}$ ACh sets up a local response at the height of depolarization; a, after a stronger concentration of ACh a spike is initiated and following it is a similar local response as above. b, saline application. Time scale: 1 d.v. = 10 msec. (see text).

A critical depolarization of the muscle membrane by the endplate potential (e.p.p.) (18, 25) or by an applied constant pulse (27) is known to set up muscle impulses. It is safe to assume that a similarly critical depolarization takes place here after application of ACh or the other drugs (see discussion). This view is further supported by the effect of subthreshold concentrations as seen in Fig. 2Ab. There a slow negative potential only is set up. With increase of concentrations larger potentials are set up until at a critical value a spike arises at the height of the potential. The depolarizing action of the drugs does not cease after the impulses have been set up, but decays slowly over many seconds as can be shown by recording with an amplifier of a long time constant. This is presumably due to the slow dissipation of the drug action.

With concentrations near threshold frequently small monophasic potentials (abortive impulses) have been recorded at the endplate region (Fig. 2Bc). A slightly higher concentration sets up a propagated impulse and following it is a small potential similar to that seen above. (Fig. 2Ba). Such local responses can be set up in isolated normal muscle fibres by electric

stimulation or in relatively refractory muscle by a nerve impulse (26, 27). Immediately after a spike (Fig. 2A) the potential returns to the original base line only and the negativity is then built up again. This seems to be analogous to the 'collapse' of the endplate potential, or of an applied catelectrotonic potential, during the muscle spike and to its rebuilding after the refractoriness has passed off (18, 27).

When a train of impulses is set up at the endplate as in Fig. 2A, 3c, and

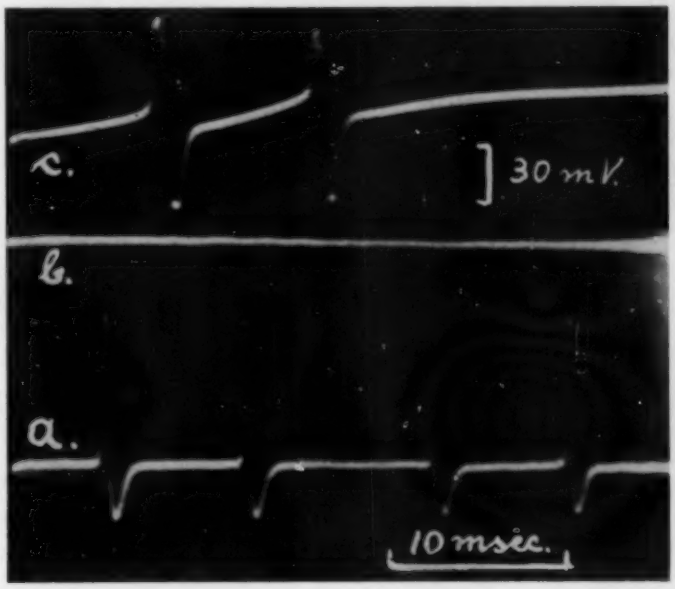


FIG. 3. Potentials recorded from nerve-muscle fibre preparation. c, acetylcholine applied to endplate while the recording is done from point of application (as fig. 1, but faster sweep). a, impulses initiated at endplate are recorded 5–6 mm. away from it. Note the absence of a negative potential preceding or following the spikes. b, base line.

the recording is done a few mm. away from the point of application, no negative potential precedes or follows the spikes (Fig. 3a); only 'simple' propagating muscle impulses are recorded and they are identical whether set up by nerve stimulation, electrical stimulation or drug application.

Threshold concentrations varied considerably with all the drugs, probably depending on the condition of the individual fibre and on the accuracy of application to the endplate. ACh and nicotine thresholds were at about $10^{-6}$ while the caffeine threshold was mostly 10 times lower. 0.22–0.45 per cent KCl diluted in Ringer from an isotonic stock solution gave usually threshold excitation. At times no propagated impulses could be set up by local caffeine application, and with high concentrations ($10^{-3}$) only contracture around the endplate region resulted. After each application the fibres were "washed" by applying drops of Ringer which could later be removed by a small pipette. But even so, after several applications of the drugs, frequently no further impulses could be set up. After lowering the preparations into saline the fibres usually recovered in a few minutes. Between applica-

tions the size of the spikes, set up by nerve stimulation, was frequently recorded. When the whole muscle fibre was immersed into 0.22 per cent KCl nerve stimulation became ineffective after 1 or 2 min. After lifting part of the preparation, comprising the nerve-muscle junction, into normal saline, the gradual recovery could be observed under the microscope. Nerve stimulation is followed first by a local contraction around the junctional region, then the contraction may gradually spread along the rest of the fibre which is not immersed, while the part in KCl does not contract. This shows that the process of neuromuscular transmission recovers before the muscle is able to conduct impulses. It also suggests that abolition of indirect excitability after KCl application was primarily due to the effects of potassium on the muscle fibre itself and not on the process of neuromuscular transmission.

A dose of curarine which blocked neuromuscular transmission diminished the sensitivity of the endplate at least 10 to 100 times to ACh, nicotine and caffeine. Sometimes no impulses at all could be initiated and doses such as $10^{-3}$ ACh set up only local contractures when applied to the endplate region. Usually the sensitivity could be restored after soaking the preparation in normal saline. The threshold concentration of KCl was frequently increased by curarine but to a much smaller degree than with the other drugs.

Two sartorius preparations were isolated together with the ventral roots. Stimulation of the ventral roots resulted in full contraction of the muscle and a normal action potential could be recorded from it. During application to the endplates of ACh, KCl and nicotine in concentrations well above threshold for setting up repetitive responses, no potentials could be recorded from the ventral roots. It can be concluded therefore, that the motor nerve endings of frog's muscle are not excited by these drugs. In cats, however, nerve discharges following injection of ACh were found by Masland and Wigton (31).

(2) *Change of "resting potential" during drug application.* It seemed of interest to record changes caused by long lasting application of drugs to different parts of the whole sartorius or to single muscle fibres. The preparation, set up vertically, was lowered to a required degree into the test solution. All the potential changes thus measured are caused by the depolarizing action of the drugs on the muscle membrane and are relative to the lead on the 'normal' muscle part in paraffin (cf. Method). At different intervals after immersing part of the muscle into the test solution changes in potential are measured.

No appreciable potential, (less than 1 mV) appeared when the endplate-free part of a nerve muscle fibre preparation was lowered into a threshold concentration of ACh for 10–15 min. (Threshold concentration of a drug was the concentration which set up impulses at the endplate when applied locally by a fine glass-hook.) However, when the endplate reached the test solution, a train of impulses was set up and a potential of several millivolts lasting for at least 10 min. could be recorded at this spot. When a muscle

fibre was lowered into 0.22 or 0.45 per cent KCl solution an immediate potential change was caused and became constant in 2–3 min. This occurred wherever KCl surrounded the fibre. No difference between endplate and endplate free parts was noticed. Whenever this potential exceeded a few millivolts, the propagating spike was diminished or abolished altogether (cf. similar findings in squid giant axons where true resting potentials across the membrane could be measured, [16]).

Also in the whole sartorius preparation the depolarizing action of ACh, nicotine, and usually caffeine could be well demonstrated. When the pelvic 2–3 mm. of the muscle was lowered into the test solution of just threshold concentration, no potential developed there, and no contraction at all was noticed. With further immersion of the muscle, however, fibrillation was set up and persisted for several seconds. At the same time a potential developed, reaching about 8–10 mV in 15 min. (cf. Fig. 4a). Sometimes this potential was very small (1–2 mV) and it always varied at different spots on the muscle. Muscles in which a threshold concentration of ACh caused a very small depolarization were sometimes immersed into an ACh solution of $10^{-3}$ concentration. 10–20 mV could then be found at the nervous part while little (1–2 mV) or no potential was recorded at the pelvic end. The small size or absence of potentials at the pelvic end and the varying size on the nervous part strongly suggests a dependence on endplate foci along the muscle. This was further confirmed by the action of curarine, diminishing or even abolishing these depolarization potentials to all the drugs except KCl. Such an opposing action of curarine to ACh depolarization in muscle has been described by Cowan (15) and the curarine-nicotine antagonism is well-known since Langley's experiments (28).

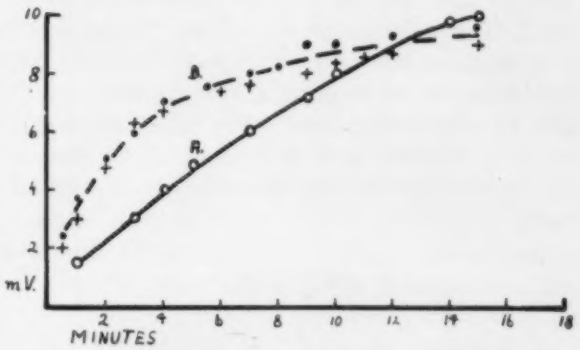


FIG. 4. Depolarization of the whole isolated sartorius. A. After immersing the 'nervous' part into $10^{-6}$ ACh. (When the pelvic end alone is lowered into ACh no potential develops.) B. Muscle lowered into 0.22 per cent potassium. Full circles: depolarization at the pelvic end. Crosses: depolarization at the 'nervous' part.

The effect of eserine in potentiating the local negative potentials set up by ACh was striking in some experiments, but very small or even absent in others. Part of the preparation was lowered into ACh ($10^{-5}$ or $10^{-6}$) for 10–15 min., then the muscle was washed with Ringer until the potential at the nervous part of the muscle disappeared. After placing the preparation into $10^{-5}$ eserine-Ringer for ½ hour the muscle was lowered into an eserine-ACh

test solution and the developing potential was measured again. Eserine had little or no effect on the nicotine and caffeine potentials.

In view of the selective excitatory action of KCl on the endplate, a difference in its depolarizing effect on the nervous part or pelvic part of the sartorius might be suspected. Two methods were employed in this investigation. (a) The muscles were immersed into different concentrations (0.22, 0.45 and 0.89 per cent) of KCl, the pelvic end first and then the nervous part while the development of the depolarization potential was plotted. Thus Fig. 4B shows the potential developed at the pelvic end (full circles) and at the "nervous" part (crosses) after immersion into 0.22 per cent of KCl. The differences do not seem significant. (b) The pelvic 2–3 mm. and 5–6 mm. of the tibial end were lowered simultaneously into two separate small dishes containing the same concentration of KCl; into these dipped the two recording electrodes. The two small dishes were in a big dish containing paraffin and were bridged by the middle of the sartorius, held up in the paraffin by two glass-hooks. Potential differences of a few millivolts were sometimes found over the first 10 min. but they usually disappeared after 20 min. Curarine did not alter the potentials in any way comparable with its striking effect on the other drugs.

### B. CHRONICALLY DENERVATED MUSCLE

After about two weeks the sensitivity of denervated muscle is considerably increased not only to ACh (6, 10), but also to nicotine and caffeine application. The KCl sensitivity, however, is not appreciably increased if compared with that of the not denervated leg of the frog. After denervation from 7 weeks upwards concentrations of the drugs as low as $10^{-10}$ or $10^{-11}$ set up impulses at the endplate regions and even a slight mechanical stimulus can initiate bursts of impulses in such a denervated sartorius. The muscle is practically on the verge of the spontaneous activity which was recently observed by Reid (32).

The isolated muscle fibre preparation gave a good opportunity for testing the sensitivity of the endplate-free regions in denervated muscle. No endplates can be observed in such preparations and therefore the whole length of the muscle fibre had to be tested. With some parts of the fibre the threshold concentration to set up impulses was 100 to 1000 times higher than with other parts. It seems safe to assume that these less excitable regions of muscle fibres are endplate-free regions. This is strongly supported by the invariably lower excitability at the pelvic end of the denervated sartorius. For example, in some instances the ACh sensitivity was increased on the 'nervous' part of the muscle to $10^{-11}$ as compared with $10^{-6}$ on the normal side. With careful application to the pelvic 1–2 mm. of the muscle in some of these experiments the threshold concentration was $10^{-7}$ or $10^{-8}$. Differences in sensitivity even to mechanical stimulation with a small glass rod or platinum loop were quite marked between the pelvic end and the rest of the sartorius especially after denervation of more than 7–8 weeks.

As in normal muscle, practically no depolarization occurs at the pelvic end of the whole sartorius if immersed into concentrations of ACh, nicotine or caffeine 10 to 100 times above threshold, while with a just threshold concentration a potential develops at the 'nervous' part of the muscle. A paralytic dose of curarine diminishes the depolarization and also raises the threshold at which locally applied drugs set up impulses. Again, the KCl threshold is not appreciably affected by curarine.

These findings show that the difference in the depolarizing action of drugs on the endplates and the rest of the muscle also persists in chronically denervated muscle.

## DISCUSSION

Chemical depolarization of membranes has been frequently observed. The depolarizing action of potassium on the muscle membrane is well known and a powerful effect of acetylcholine (ACh) in reducing injury potentials has been shown by Cowan (15). When diluted alcohol or veratrine are applied to crab nerve the membrane becomes depolarized and a train of impulses is set up (1, 2). A similar stimulating effect is caused by potassium application (22).

At the endplate region a local potential can be produced by drug application (Fig. 2Ab) and may reach a critical value and then set up either local (Fig. 2Bc) or propagated responses (Fig. 2 and 3). The same sequence of events, as above, can be seen after application of a catelectro-tonic potential, produced by constant current pulses, to isolated muscle fibres (27). Further, subthreshold depolarization and local responses can be set up by the endplate potential in curarized and refractory muscle (20, 25, 26). Thus the excitatory action of drugs is similar to an applied current pulse and to the 'transmitter action' underlying the endplate potential. It seems clear from the present experiments that depolarization of the muscle membrane is caused by certain drugs at the point of application.

The specific excitatory action of ACh, nicotine and caffeine at the endplate is well in line with the selective depolarizing effect at that region. It shows that the endplate is a specialized part in the nerve-muscle system and has a different chemical excitability from nerve or muscle. Distinct membrane properties of the endplate region which may account for this different excitability have not yet been found. Electrically a special excitability, as Lucas's $\beta$ excitability could not be shown on the whole muscle (33). The fact, however, that the $\beta$ excitability can be reduced or abolished by curarine is very suggestive that Lucas was stimulating the endplate region, as neither muscle or nerve is appreciably affected by curarine. When depolarization is caused by application of drugs, impulses are set up at the endplate region at a critical threshold level, in agreement with other excitable membranes (23). The height of the depolarization potential was in the present experiments between 20 and 40 per cent of the subsequent spikes. It is possible that at the endplate itself the membrane becomes completely depolarized

similar to the depolarization caused by the 'transmitter action' after nerve stimulation (25, 27). In the present investigation the recording electrodes made contact with the big area and this would certainly diminish the size of the potential recorded from the endplate region.

The failure of KCl to set up impulses at the endplate-free regions of muscle is somewhat surprising as it depolarizes the muscle membrane similarly at all points and would be expected to have the same effect as on nerve, for instance on crab nerve (22). Also veratrine resembles potassium in that equally strong after-potentials appeared on the 'nervous' and pelvic part of locally veratrinized muscle, though most of the repetitive after discharges originated from the innervated part. (Katz, unpublished observations). The above results could possibly be explained if the rate of depolarization by potassium is actually slower off the endplate, but the difference might not be recorded on account of diffuse leading. An additional excitation might occur at the endplate by liberation of ACh by potassium, just as is observed in the superior cervical ganglion (21, 8).

A selective effect of ACh in initiating the discharge of impulses from the endplate could be expected from the experiments of various authors (9, 5, 6, 13) (for an extensive review see Brown, 7). Such an excitability could be well explained by the specific depolarizing action as found in the present experiments. This might also apply to ganglion cells which respond to much lower concentrations of ACh than preganglionic or postganglionic fibres (4). Accommodation, however, seems much quicker in muscle than in ganglion cells. These latter continue to discharge for many minutes provided the ACh concentration of the perfusion fluid is maintained (4). In muscle only a few bursts of impulses are set up if the endplate region is immersed into a bath of threshold concentration of ACh.

The initiation of slow waves by caffeine in frog's brain (28) might also be due to a selective depolarizing action of this drug.

It is not possible to determine whether impulses can be set up by application of drugs at endplate-free regions of denervated muscle. In some isolated fibre preparations the muscle seemed excitable everywhere, but one cannot exclude the spread of the drug to an endplate near the point of application. It also can not be established where the endplates are and how many there are on a denervated muscle fibre preparation. The evidence from the whole normal or denervated sartorius supports the view that no impulses are set up at the endplate-free regions. Those apparently arising there are probably due to the spread of the drug to some endplates nearby. If impulses were set up at the pelvic end of the muscle an appreciable depolarization of the membrane should appear there. This could not be observed after ACh, nicotine and caffeine had been applied to the pelvic end of the sartorius. The outstanding change in the muscle following denervation seems to be the greatly increased excitability of the endplates. This is of interest, as it suggests strongly that spontaneous fibrillation is entirely due to impulses arising at the most excitable part of the muscle, that is at the endplate.

Du Bois-Reymond (17) and others state that no potential differences exist between two uninjured parts of a muscle (cf. for extensive discussion Biedermann, 3). A potential difference is observed only when the muscle fibres are cut, crushed or otherwise injured. However, potential differences have been found between different parts of the muscle surface in frog, increasing when the recording electrodes were moved further apart (11). Buchthal and Lindhard (12) have also found that in lizards a potential normally exists between the endplate and the adjacent region of the muscle fibre. The endplate was either positive or negative relative to the fibre and sometimes very great potential differences were found (cf. 14) In the present experiments these large potential differences could not be found in frog's muscle. It is difficult however, to exclude small potential differences with the presently employed fluid electrodes, because potentials confined to the small area of the endplate itself (0.1–0.2 mm.) may not be recorded unless a very well defined contact with that area is made.

## Summary

Properties of the endplate and endplate-free regions were investigated in single nerve-muscle fibre preparations of the M. adductor longus and in isolated sartorius muscles of Australian frogs (*Hyla aurea*).

1. Acetylcholine, nicotine and caffeine set up impulses by depolarizing the muscle membrane at the endplate region. None of the drugs appreciably depolarizes endplate-free parts of muscle, or sets up impulses there.

2. Potassium initiates impulses at the endplate region only, but no striking difference could be detected between the depolarizing action at or off the endplate.

3. Curarine opposes the depolarization and excitation caused by the drugs, except potassium.

4. The sensitivity at the endplates to acetylcholine, nicotine and caffeine in chronically denervated muscles (3–11 weeks) is increased from 1,000 to 100,000 times and is diminished by curarine. The selective depolarization of the endplate region and the lack of depolarization at the endplate-free regions pertains also to denervated muscle.

5. No appreciable potential differences are found in normal uninjured muscle along the outside of isolated fibres. No evidence was obtained of potential gradients around the neuromuscular junction.

ACKNOWLEDGMENT

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# REFLEX ACTION IN RELATION TO PATTERN AND PERIPHERAL SOURCE OF AFFERENT STIMULATION

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THE SEGMENTAL reflex discharge obtained by single shock stimulation of a dorsal root while recording from the ventral root of the same segment displays a prominent initial peak, which is transmitted through arcs of two neurons, followed by an irregular discharge elevation of some 10 msec. duration, all of which is presumed to represent discharges mediated through more complex arcs involving interneurons (7, 9, 11). No concerted effort has been made to relate the type of reflex discharge obtained to the size (*i.e.* the fiber spectrum) of the dorsal root volley employed, although considerable information is at hand concerning the fiber sizes and conduction rates of the various afferent fibers entering into the constitution of the nerves and dorsal roots (3–6, 10, 13). The experiments presented here have as their aim, therefore, the correlation of the central effect of an afferent volley, in terms of reflex discharge, facilitation and inhibition, with the fiber size range and peripheral origin of that afferent volley.

The afferent fibers involved in the present experiments are those which contribute to the first spike elevation in the dorsal root. This elevation is impressed upon the recording system essentially by the A fibers of the dorsal root exclusive of those belonging to the delta sub-group. The action of the delta fibers has not been considered in the present experiments. It should be noted however that the addition of delta fibers to the already active fibers of faster conduction and larger diameter, contributes immeasurably to the occurrence of afterdischarge in the resulting reflexes (14, cf. also 1, p. 565).

By recording directly the volley evoked in a given dorsal root by a single shock, and the results of that sized volley, it is possible to relate the intensity of end action and response to the size of the causal afferent volley. In the accompanying graphs the size of the afferent volleys is plotted along the abscissa, and is expressed in terms of the maximum attainable initial spike potential. The size of the initial (two-neuron-arc) reflex volley (Fig. 1) and the degree of facilitation and inhibition (Fig. 3) of such reflex discharges by appropriate afferent volleys are plotted as ordinates, and are expressed in per cent of the maximum attainable effect. In practice, control observations of the dorsal root spike potential were taken before and after each series of experimental observations. The experimental series in each case was sufficiently long to neutralize the characteristic fluctuations in responsiveness of the 'resting' cord.

The first sacral (S1) segmental reflex was employed for the most part to study the discharge of motoneurons, or as a test reflex response to gauge the effectiveness of subliminal excitation and inhibition. For facilitation of a

test two-neuron-arc reflex a small filament of the L7 or S1 dorsal root was segregated for conditioning stimulation. The size of the selected filament was such that a maximal volley evoked within it would not secure a two-neuron-arc reflex discharge within the motoneuron pool to be tested. For inhibition the whole, or some fraction, of the L6 dorsal root was stimulated to secure the 'inhibitory' volley (cf. 8).

*The discharge of motoneurons.* Between the size of a dorsal root (presynaptic) volley and the size of the discharge evoked from motoneurons by

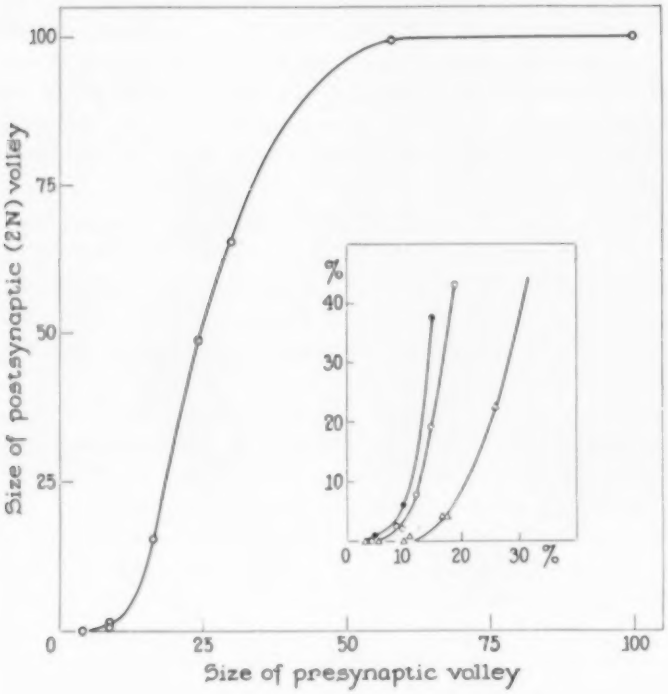


FIG. 1. Dorsal root-ventral root reflex preparation. The size of the two-neuron-arc (2N) reflex discharge is plotted against the size of the afferent volley evoking the reflex. Inset: the initial portions of similar curves from other experiments to illustrate the variation in onset of the reflex discharge from one experiment to another. The curves are constructed from observations such as those recorded in Fig. 2. Full description in text.

the direct action of that volley, a definite relation holds. The relation is found to follow a sigmoid curve. As may be seen in the experiment plotted in Fig. 1, the reflex volley begins to appear only after the dorsal root spike potential has reached 7–8 per cent of its maximum. The inset of Fig. 1 illustrates the variation encountered from one experiment to another. Once a discharge zone is secured within the segmental pool the size of the reflex postsynaptic volley increases rapidly and apparently linearly with increase in the presynaptic volley until the latter volley is approximately 50 per cent maximal. The reflex volley is then from 90–95 per cent complete. Bearing in mind the relationship between spike height, fiber size and conduction rate (5) it is evident that stimulation of considerably less than one half of the fibers contributing to the initial spike potential of the dorsal root secures very nearly the maximum attainable two-neuron-arc postsynaptic volley.

Renshaw (11) has estimated that A fibers conducting at velocities of no less than two-thirds maximal account for the initial reflex discharges. The failure of the reflex volley to increase further as more dorsal root fibers are recruited into the causal volley is due not to a saturation of the motor pool, but is due to the failure of the smaller fibers to reach the motoneurons at all, for it is a commonplace observation that the addition of but a few fibers of the appropriate size range to the stimulated bundle results in a further increase in the postsynaptic volley.

Figure 2 illustrates sample records from a series such as that employed to construct the graph of Fig. 1. The records were obtained from the S1

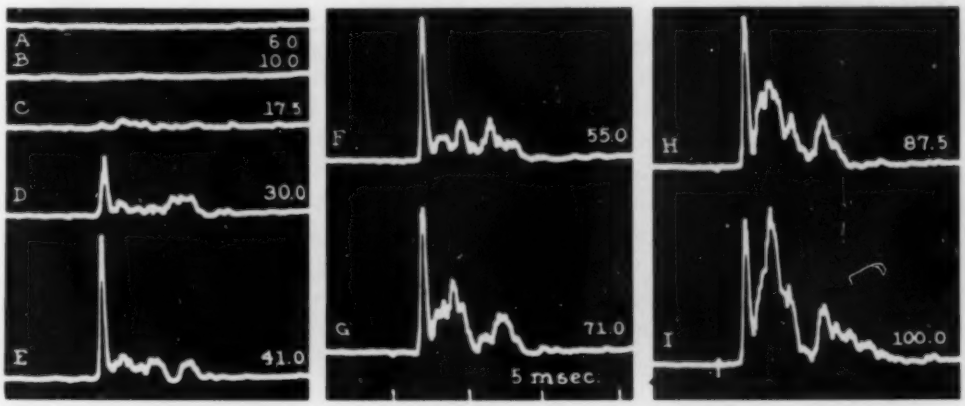


FIG. 2. Dorsal root-ventral reflex discharges from the first sacral segment. The successive records illustrate the growth of the reflex discharge as the afferent (dorsal root) volley is increased in size. The figures to the right hand of each observation give the relative size of the afferent volley employed for that observation. Time = 5 msec.

ventral root at successively increasing strengths of dorsal root stimulation. The figures to the right of each observation give the amplitude of the dorsal root volley, in per cent of maximum, obtaining for that observation. A two-neuron-arc discharge is first encountered in Fig. 2B, wherein the afferent volley is 10 per cent of maximum. The two-neuron-arc discharge is maximal in 2E, for which observation the dorsal root volley was but 41 per cent of maximum.

Although Fig. 2 shows, with reference to two-neuron-arc discharges, the type of observation employed in constructing the curve of Fig. 1, it is presented primarily to illustrate the relation between the size of a dorsal root volley and the amount of motoneuron discharge secured through the more complex segmental reflex arcs. Some discharge pertaining to the more complex arcs is apparently realized as soon as a two-neuron-arc discharge is discernible, but intense development of the multineuron-arc discharge occurs only as the dorsal root volley increases beyond 50 per cent of its maximal size towards maximality. Estimates based on the area enclosed by

the action potential of the multineuron-arc discharges suggests that such discharges become 50 per cent of maximum only after the dorsal root volley is 70–85 per cent maximal. The discharges increase rapidly thereafter. Thus, within the fiber group represented by the initial spike potential of the dorsal root, the lowest threshold members contribute heavily to the two-neuron-arc responses while the higher threshold members account in large measure for the multineuron-arc discharges.

One factor to be considered in evaluating the observations on multineuron-arc discharges is the possible effect of the rapidly developing two-neuron-arc discharge. As the two-neuron-arc discharge increases it might be expected to occlude to some extent the ensuing multineuron-arc discharges. However, after the stage represented in Fig. 2E, the factor of occlusion need not be further considered as the two-neuron-arc reflex preempts no more motoneurons to its service, whereas the major intensification of the later discharges occurs only after this stage is reached. Occlusion then is not the important factor. It appears rather that the central connections of the higher threshold fibers are limited to interneurons.

*Facilitation and inhibition.* The relationships that have been described show how the motoneurons respond to the end action of the dorsal root fibers. It is possible, by utilizing the variation induced by a measured presynaptic volley in the response to a standard test excitation, to measure more directly the end action of presynaptic fibers. Inasmuch as subliminal action is measured, the tests are more subtle than those based purely on discharge response. Since inhibition, in contrast to excitation, must inevitably be assessed in terms of a standard test excitation, the procedure has the advantage of providing a quantitative measure of direct inhibition, and for a comparison between the direct excitatory and direct inhibitory end effects of appropriately selected dorsal root volleys.

In Fig. 3 are plotted the direct excitatory (circles) and direct inhibitory (dots) end effect of appropriate dorsal root volleys upon standard testing two-neuron-arc reflex responses. The intensity of facilitation or inhibition is expressed as a function of the size of the conditioning volley in each case. As in the example provided by reflex discharge, a definite relationship holds between the size of the causal dorsal root volley and the intensity of its end effect (excitatory or inhibitory) in the motor nucleus. The relationship is similar for the two opposed actions. The excitation or inhibition of motoneurons as the case may be, is initially exerted by the lowest threshold fibers in the dorsal root. By the time that the dorsal root volley reaches 50 per cent of maximum, the excitatory or inhibitory action of that volley on the test two-neuron-arc discharge is approximately 90 per cent complete. The virtual identity of the excitatory and inhibitory curves indicates that dorsal root fibers of identical conduction properties are involved in both instances. Moreover it is clear that the lowest threshold fibers in both cases exert most of the end effect.

The simplicity of the two curves suggests that the excitatory and in-

hibitory end effects are separate and not intermingled under the anatomical and temporal restrictions employed in these experiments. However, the very identity of these curves makes it conceivable that the two end effects as measured in Fig. 3 could be intermingled and yet appear as pure processes. For instance, if the two opposed processes were intermingled and were to maintain a constant proportionality at all strengths of the causal dorsal root volley, then the resulting experimental curve, representing the difference, would indicate only the more powerful, and would differ in no way from

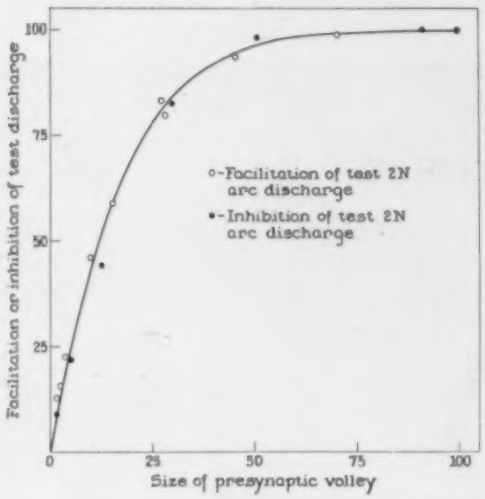


FIG. 3. A comparison between the development of direct excitation and direct inhibition of motoneurons. Circles: the course of facilitation of a standard test two-neuron-arc reflex plotted against the size of the conditioning dorsal root volley. Each point represents the difference between the facilitated response and the control response expressed in per cent of the difference obtained by use of the maximal conditioning volley. Dots: the course of direct inhibition similarly plotted, but from another experiment. Each point represents the difference between the control response and the inhibited response expressed in per cent of the inhibition obtained by use of the maximal conditioning volley. Further details in text.

the curves to be expected from exquisitely segregated excitatory and inhibitory processes.

Simple and superimposable curves, such as those illustrated in Fig. 3, would not be obtained if excitatory and inhibitory action differed in any way related to the fiber population occupied by the causal afferent volleys. Therefore, the fibers which mediate direct excitation and direct inhibition to the motoneurons are indistinguishable on a functional basis. There is, in consequence, no reason to suppose that a special group of fibers is employed in each case.

*Relation between subliminal fringe and discharge zone in simplest reflex system.* Figure 4 represents diagrammatically the relation between the subliminal fringe and the discharge zone within a motoneuron pool upon the occasion of direct excitation through dorsal root collaterals. The diagram is based on the experiments described in connection with Fig. 1 and 3.

The initial upward concavity, present in the discharge curve (cf. Fig. 1) but absent in the excitation curve (cf. Fig. 3) undoubtedly reflects the need for summation within the motor nucleus before discharge may occur. Accordingly in the 'resting' preparation a synchronous volley of considerable dimensions must reach the motor nucleus in order that a firing zone may be

established. It is unlikely therefore that any significant number of motoneurons are close to or at threshold in the 'resting' pool, for if there were, the first afferent impulses to enter the pool should secure a postsynaptic discharge. The resulting discharge curve would then approximate in form that of the facilitation curve.

The diagram of Fig. 4, dealing as it does with synchronous afferent excitation and two-neuron-arc discharges does not contain a time parameter, for time is fixed and intensity only need be considered. When, however, excitation occurs within the motor nucleus as the result of a diffuse influx of impulses, rising and falling in intensity over a number of milliseconds, as in the instance presented by long spinal reflex excitation (9), the changes plotted in Fig. 4 as a function of intensity of presynaptic excitation, will take place with time as an added parameter. In effect, events will progress initially to the right along the abcissa as the impulse bombardment increases in intensity, and subsequently to the left as the impulse bombardment wanes. On this schema the motoneuron discharge realized as the result of a diffuse waxing and waning excitation should appear only after a considerable nuclear delay occasioned by the degree of excitation necessary to secure a discharge zone, and should cease considerably before the presynaptic bombardment, aided unquestionably also by the development of subnormality among the motoneurons. In short the motoneuron discharge should be considerably synchronized relative to the causal synaptic barrage. This is precisely what does occur, for instance, when lumbar or sacral motoneurons are excited by activity transmitted through the long spinal reflex system (9).

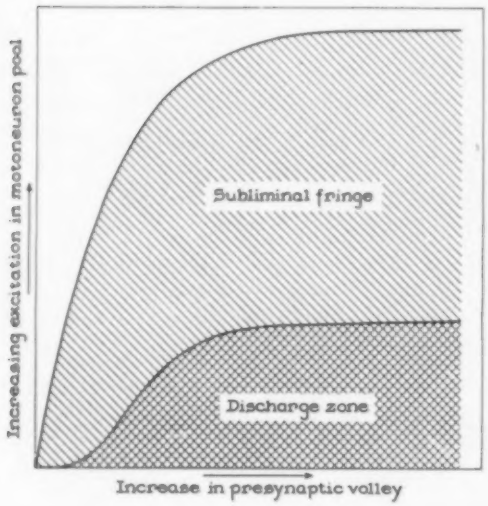


FIG. 4. A diagram based on Fig. 1 and 3, to illustrate the relation between subliminal fringe and discharge zone in a motoneuron pool activated by dorsal root volleys.

*Peripheral origin of afferent fibers contributing to two-neuron-arc, and to later reflex discharges.* From a study of Fig. 1, 2, and 3 it is seen that the low threshold dorsal root fibers mediate direct effects to the motoneurons, whereas the higher threshold fibers act indirectly through internuncial relays. Since the low threshold fibers are those of largest diameter, and those which conduct impulses at the greatest velocities, it follows that the large, rapidly conducting fibers of the dorsal root are responsible for direct action on the motoneurons.

The most rapidly conducting afferent fibers have a conduction velocity

of approximately 115–120 M/sec. Employing the factor of six relating conduction rate to fiber size (6), the largest afferent fibers would be approximately 20$\mu$ in diameter, which is in full agreement with the histological observations of Sherrington (13). The present observations and those of Renshaw (11) are in agreement that the upper third of the fiber spectrum accounts substantially for the two-neuron-arc afferent fibers. This would place the bulk of the afferent fibers establishing direct connection with motoneurons in the range above 12$\mu$–13$\mu$ in diameter.

It is well known that cutaneous nerves contain but few fibers greater than 12$\mu$ in diameter (2, 3, 5), so it is unlikely that the afferent fibers in cutaneous nerves form direct connections with the motoneurons, though there can be no doubt that these fibers contribute heavily to the multineuron-arc reflexes. On the other hand the conspicuous pile in the fiber spectrum of 'demotored' muscle nerves, i.e. motor nerves from which the motor fibers have been degenerated by ventral root section, lies in the diameter range above approximately 12$\mu$ (2, 10, 13), whereas the number of fibers between 12$\mu$ and 6$\mu$ is scant. It follows from these several observations that the two-neuron-arc-reflex discharge should result from stimulation of the afferent fibers from muscles, but not from the stimulation of cutaneous nerves, while stimulation of the cutaneous nerves should result in a profound reflex discharge of greater central latency. This conclusion has been put to test in the following manner and established as correct. The sural nerve and the nerves to the gastrocnemius muscle are utilized to provide a cutaneous afferent volley and a muscle afferent volley respectively. The L7 and S1 ventral roots are severed to provide a means of recording the reflex discharges, and incidentally to avoid the untoward effects of antidromic volleys sweeping from the gastrocnemius nerve into the motoneurons (cf. 12).

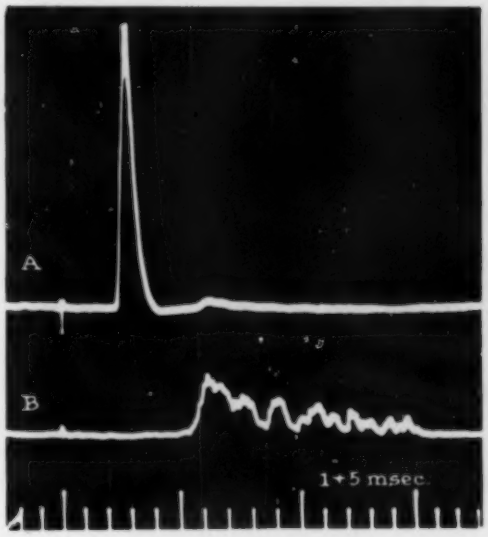


FIG. 5. Reflex discharges recorded in a ventral root and resulting from maximal afferent volleys in (A) the nerves to the gastrocnemius muscle, and (B) the sural nerve. The total conduction distance for the two reflexes, and the amplification employed are comparable. Further description in text.

Figure 5A presents the reflex discharge recorded from S1 motoneurons and evoked by an afferent volley in the nerves to the gastrocnemius muscle. The feature in this record is the two-neuron-arc discharge appearing with a total latency of 2.4 msec., which is only sufficient to account for conduction

and a single synaptic relay. There is a small delayed discharge appearing with a total latency approaching 6 msec. In Fig. 4B is recorded the reflex discharge obtained in the ventral root by stimulation of the sural nerve. In this record the earliest reflex discharge appears with a total latency of about 5 msec. Since, in its afferent course the sural nerve-ventral root reflex must travel in fibers of slower conduction than does the gastrocnemius nerve-ventral root reflex, some of the added latency may be accomodated as added conduction time. The time accounted for by differential conduction rates would be less than 0.5 msec., which means that the sural nerve-ventral root reflex has the longer central latency by about 2.0 msec. Furthermore, the sural nerve-ventral root reflex discharge is irregular and has a duration of about 10 msec., which features are characteristic of the later portions of the familiar dorsal root-ventral root reflex. Thus a segregation of afferent fibers according to their origin in muscle or in the skin results in a high degree of segregation of the two-neuron-arc and later reflex discharges. The two-neuron-arc connections are placed at the service of the afferent influx from muscle; the afferent impulses from cutaneous areas are routed through the more complex and diffuse internuncial systems.

In consideration of the identity of afferent fiber range mediating excitation and inhibition directly to the motoneurons (Fig. 3), it follows that the dorsal root fibers mediating direct inhibition to the motoneurons have their origin within the muscle afferent system.

## Summary

Two-neuron-arc reflex discharges in the dorsal root-ventral root reflex are secured by stimulation of the lowest threshold fibers of the dorsal root. Stimulation of the higher threshold fibers is primarily responsible for the multineuron-arc reflex discharges.

The fibers mediating direct excitation and direct inhibition to the motoneurons are functionally indistinguishable in the dorsal root.

Afferent fibers of the size range having direct connection with the motoneurons are found in significant numbers only in muscle nerves. The group of afferent fibers which accounts heavily for connections to interneurons is prominent in cutaneous nerves, but poorly represented among the muscle afferent fibers (2–5, 10, 13). Utilizing this information it is possible to effect a high degree of separation of the two-neuron-arc and multineuron-arc reflex discharges by employing a muscle nerve and a cutaneous nerve respectively for afferent stimulation.

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# FUNCTIONAL ORGANIZATION OF TEMPORAL LOBE OF MONKEY (*MACACA MULATTA*) AND CHIMPANZEE (*PAN SATYRUS*)

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## INTRODUCTION

EXPERIMENTS on the sensory and adjacent cortex of the monkey and chimpanzee (2, 12, 3) frequently necessitated controls by placing electrodes or applying strychnine to other parts of the cortex. The incidental information so acquired gave valuable clues for further experimentation. The functional organization of the occipital lobe, centering around the primary visual cortex, has already been reported (6). It was hoped that, by concentrating attention upon the temporal lobe, it might similarly be possible to make out the functional organization centering around the primary auditory cortex. For that reason the present experiments were undertaken.

The temporal lobe, as that term is used in anatomy, is completely covered by isocortex. It does not include the pyriform area and the hippocampal formations—*i.e.*, the cornu ammonis proper and the entorhinal and adjacent areas. On cytoarchitectural grounds Campbell (10) divided this part of the brain in man, chimpanzee and orang into an auditosensory, an auditopsychic and a common temporal area. Brodmann (7, 8) sub-divided in man the common temporal area into areas 22, 21, 20 and 38, in the cercopithecus into 22, 21 and 20. Mauss (18, 19) recognized a temporo-polar area in the orang and gibbon but not in the cercopithecus. v. Economo and Koskinas (14) divided the temporal lobe of man much in the same way as Brodmann. Brodmann and Mauss failed to mark an auditosensory or auditopsychic area in the monkey. In man, Brodmann (7) described an area 42 as the auditosensory and an area 41 as the auditopsychic area. In a later contribution (8) he stated that the "regio supratemporalis" could be divided into four areas: 22, 41, 42, 52. Mauss recognized two areas in the orang, which he called 40 and 38, but only one in the gibbon, which he labeled 40.

The study of the thalamocortical relations in the macaque and chimpanzee (25–28) suggests a division of the temporal lobe into two major parts: (i) an area receiving radiations from the medial geniculate body, and (ii) a much larger part devoid of thalamic radiations. The first of these is the auditosensory area and covers no more than a few square centimeters on the supratemporal plane. The second of these comprises the rest of the temporal

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† National Research Council Fellow, 1940–1941.

lobe. As will be shown presently, a portion of this second part stands in functional relation to the acoustic area while the rest does not. We shall therefore divide the temporal lobe into an acoustic and a temporal sector. The precise location of the boundary between them presents one of the problems of this investigation.

## METHOD

The functional organization of the temporal lobe was investigated by the study of changes of its electrical activity in response to various stimuli. The experiments were per-

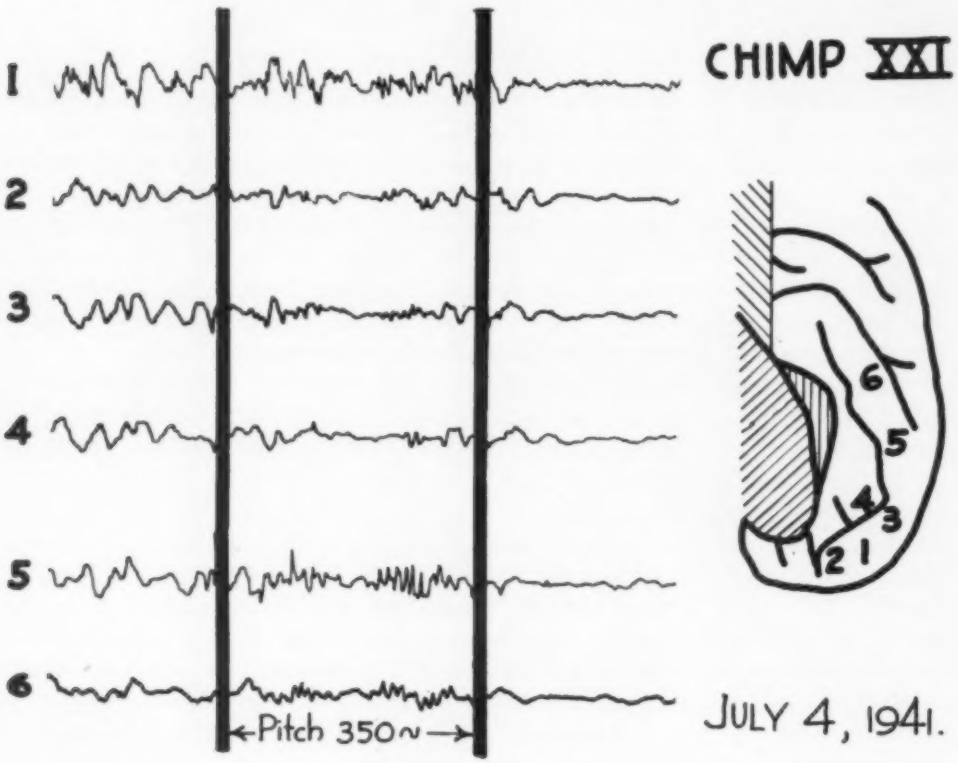



FIG. 1. Response to acoustic stimulation of supratemporal plane of chimpanzee. Sketch on right shows supratemporal plane with position of pickup electrodes. Records on left show cortical responses. Numbers 1 to 6 correspond to numbers of electrodes in unipolar pickup.

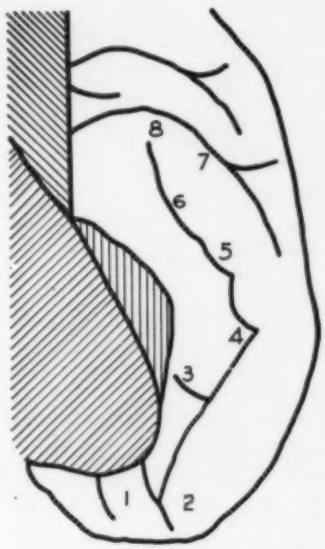
formed upon several monkeys (*Macaca mulatta*) and two chimpanzees, in each case under moderate Dial* anaesthesia. Practically the whole of one hemisphere was exposed by wide opening of the skull and dura mater. In order to reach the inferior aspect of the temporal lobe, the animal was securely fastened with its belly on a board. This board was then inverted so that the animal hung supine. After cutting the anastomotic vein of Labbé the brain had sagged out enough in about one hour to give access to its basis. In order to expose the supratemporal plane the animal was righted again and the parietal operculum was

* 0.45 cc. gr. per kg., ½ intraperitoneal, ½ intramuscular. We wish to thank Ciba for putting the Dial at our disposal.

carefully resected subpially by suction, so as to preserve the temporal pia mater and blood supply. A brain so prepared will exhibit essentially normal electrical activity for as much as 72 hours. Silver electrodes were placed at all points to be investigated. In most animals 36 electrodes were used and records made synchronously from 6 of them at a time. The technique has been described in detail in previous publications (6, 12).

## Results

1. *Acoustic sector.* In the intact brain electrical activity can be stopped or greatly reduced by any jolt or sudden sound sufficiently intense to startle the beast. Properly speaking, this abatement of activity is not an auditory




| ELECTRODES | PITCH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 100 | 200 | 400 | 800 | 1600 | 3200 | 6400 |
| 1 — 2 | O | O | O | O | O | O | O |
| 3 — 4 | ? | + | O | O | O | O | O |
| 5 — ⏚ | + | + | O | O | O | O | O |
| 6 — ⏚ | + | + | + | + | O | O | O |
| 7 — ⏚ | O | + | + | + | + | O | O |
| 8 — ⏚ | O | O | + | + | + | + | O |

TONOTOPIC ORGANIZATION
SUPRATEMPORAL PLANE ~
CHIMPANZEE XXI

Fig. 2. Tonotropic organization of the supratemporal plane of chimpanzee. At right, responses of electrodes to various pitches; at left, position of electrodes.

affair, for jolting is as effective as sound, and it affects all parts of the cortex approximately equally and approximately synchronously. A different reaction, however, is observed in the posterior part of the supratemporal plane,—that is to say, in that part which is the primary auditory cortex. From here an increase in electrical activity upon the beginning and the end of an acoustic stimulus can be observed. Figure 1 shows a typical response to a frequency of 350 cycles per sec. It shows the increased activity at the onset followed by an abeyance of activity which is part and parcel of the generalized diminution due to startle. This reaction can only be observed in the acoustic area. Occasionally, however, one can just see, synchronously with the abeyance in the acoustic area, a small disturbance appearing in the immediately adjacent cortex of the supratemporal plane. This phenomenon has never been observed on the lateral side of the temporal lobe.

It proved possible to map out a tonotopic localization in the comparatively large brain of the chimpanzee. As Fig. 2 shows, low pitches are picked up chiefly in the antero-lateral portion, and high pitches in the postero-medial portion of the acoustic area. The cortical surfaces from which responses to different stimuli were picked up overlap appreciably. Whether this is to be explained by the physical conditions of the pick-up, or by the

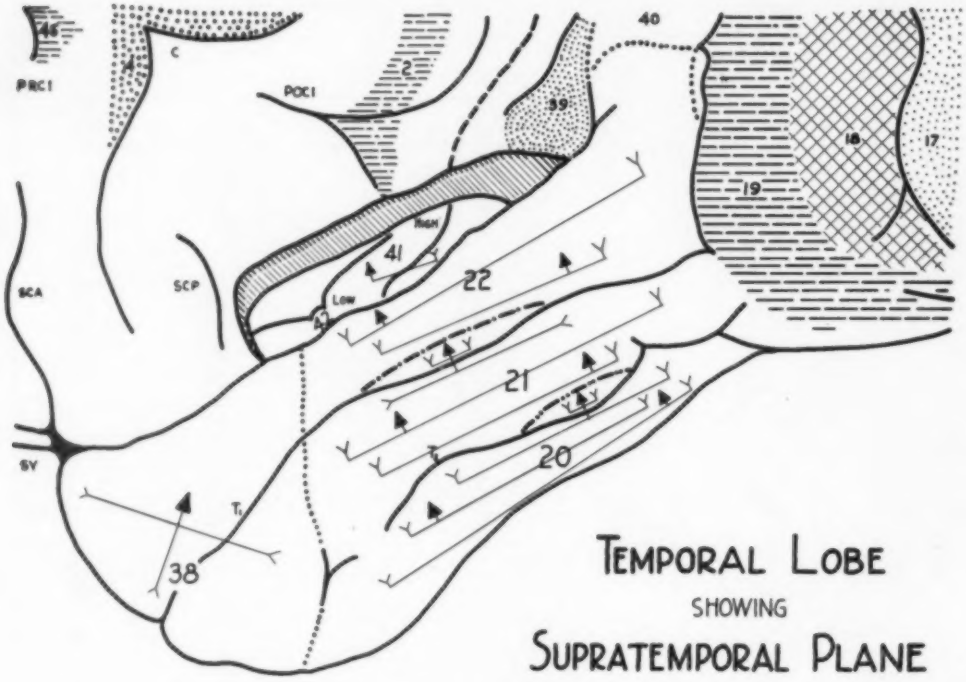



FIG. 3. "Firing" diagram of temporal lobe of chimpanzee. The supratemporal plane is exposed by resection of parietal operculum. c: sulcus centralis. poci: sulcus postcentralis inferior. prci: precentralis inferior. sca: subcentralis anterior. scp. subcentralis posterior. sy: sylvian fissure. $T_1$: sulcus temporalis superior. $T_2$: sulcus temporalis medius. The numbers correspond to Brodmann's areas. For details see text.

unavoidable background noise, or by overlapping of the fibers of the acoustic radiation, cannot be decided

When strychnine is applied locally to the primary auditory cortex, as defined by the previous experiments, spikes are propagated into area 22 on the lateral side of the temporal lobe, but nowhere else. Conversely, strychninization of area 22 causes spikes to appear in the primary auditory cortex as well as elsewhere in 22.

2. *The temporal sector*. The rest of the temporal lobe can be subdivided in both macaque and chimpanzee into three areas. Strychninization of each area "fires" all of that area but does not "fire" any point in any other area, as Fig. 3 is intended to show. The areas so mapped out correspond roughly

to Brodmann's areas 21, 20 and 38, as shown in the human brain. However, the first and second temporal sulci are no more than approximate landmarks, for in both monkey and chimpanzee areas 20 and 21 protrude slightly over the dorsal lip of the second and first temporal sulcus, respectively. Obviously, in both cases the area ventrally adjacent to the sulcus covers both of its walls.

The problem finally arose whether commissural connections between the two temporal lobes existed. In the intact animal "cross-firing" was observed only from the second temporal convulution—*i.e.*, from area 21, to the homologous loci on the other hemisphere. The appearance of strychnine spikes in the contralateral hemisphere was not abolished by cutting the corpus callosum, and it is concluded that the commissural fibers between the two temporal lobes take their course through the anterior commissure (3, 20).

As has been shown elsewhere (2, 6), area 20 receives afferent impulses travelling through the inferior longitudinal fasciculus from the parastriate area 18, and area 38 receives impulses through the uncinate fasciculus from the area orbitalis agranularis, the homologon of Brodmann's area 47 and v. Economo and Koskinas' area FFa in the human brain.

## Discussion

The work reported in this paper confirms the conception of Campbell and Walker that the temporal lobe can be divided into two sectors, an acoustic or supratemporal and a temporal sector. Our work also makes it clear that, contrary to Brodmann's statement, there is in the brain of the monkey and chimpanzee an area homologous to what Brodmann called 42 in the human brain. A koniocortex on the supratemporal plane was indeed described by Walker (25) for the macaque, and also mentioned at about the same time by v. Bonin (5) for the cebus. The minute myeloarchitectural studies by Beck (4) on the chimpanzee are hard to evaluate. Examinations of several brains of macaques and chimpanzees have shown that both primates possess an area of koniocortex on the supratemporal plane and that this koniocortex is developed to a different degree in different parts of that area, just as it was demonstrated for man in the detailed study by v. Economo and Horn (15).

The tonotopic organization found in the macaque and chimpanzee confirms Pfeifer's (22) surmise concerning the human brain to which he was led by a critical survey of clinical observations and by his study of the acoustic radiation. It is also in agreement with Walker's (25, 27) studies on the projection of the medial geniculate body on the cortex in the macaque and in the chimpanzee.

The theory advanced many years ago by Polyak (23) on the basis of careful anatomical studies has been confirmed and implemented by modern physiological research. It has been established that there is a tonotopic organization in the cochlea (11), and Walzl and Woolsey (29) have demonstrated in the cat that there is a topological representation of the cochlea

in the primary acoustic area of the cortex. After Ades and Felder (1) had defined the acoustic area of the monkey by click stimulation of that animal, Licklider and Kryter (17) found a tonotopic organization within that area, the lower frequencies activating the antero-lateral part of that region, the higher ones the postero-medial one. Our own work confirms Ades and Felder's observations on the monkey, and extends Licklider and Kryter's findings to the chimpanzee. Lashley's (16) findings in the rat would indicate that a tonotopic organization is of relatively recent phylogenetic origin.

The relation between auditory cortex and area 22 is similar to that between striate and parastriate area, which was reported on by v. Bonin, Garol and McCulloch (6)—with this difference, however, that secondary disturbances due to acoustic stimulation were never observed within area 22. They were observed, however, in the immediate vicinity of the auditosensory area—*i.e.*, in the area temporalis magnocellularis, the homologue of Brodmann's area 42 and v. Economo's area TB in the human brain. The acoustic sector can therefore be subdivided into three areas, known in the human brain as 41, 42 and 22, or TC, TB and TA respectively.

In the temporal sector, area 38 could be identified by physiological methods. It was indicated by Brodmann (7, 8) in the human brain as covering the temporal pole, but it was not given on his map of the monkey. It was found, however, by v. Bonin (5) in the cebus. Histological controls showed that the cortex covering the temporal pole in both macaque and chimpanzee differs from that covering the rest of the temporal pole in precisely the same manner in which it differs in man. The inner granular layer is much attenuated; both third and fifth layers contain mostly smaller cells, and the cell density is lower than elsewhere in the temporal lobe. It is possible to differentiate the temporo-polar area histologically as well as functionally in the brains of all gyrencephalic primates.

Our conclusion that the commissural fibers between the two temporal lobes take their course through the anterior commissure confirms what Rundles and Papez (24) and Bucy and Klüver (9) have stated on the basis of anatomical evidence. No attempt has been made, however, to cut the anterior commissure and leave the corpus callosum intact. It can not be asserted, therefore, that *all* commissural fibers between the two temporal lobes take their course through the anterior commissure.

## Conclusions

The temporal lobes of macaque and chimpanzee show the same functional organization. The lobe may be divided into two sectors: (i) an acoustic sector, consisting of the primary acoustic or auditosensory area 41, a small area 42 surrounding it, and area 22, mutually spiking each other, and (ii) a temporal sector, comprising areas 21, 20 and 38, each of which fires only locally. In the primary acoustic area of the chimpanzee tones of low frequency arrive in its antero-lateral part, tones of high frequency in its postero-medial part. Within the temporal sector the existence of a temporo-

polar area has been demonstrated in the macaque. Commissural connections between the two temporal sectors are restricted to area 21 and take their course probably through the anterior commissure.

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# LONG ASSOCIATION FIBERS IN CEREBRAL HEMISPHERES OF MONKEY AND CHIMPANZEE*

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DURING experiments on the functional organization of the cerebral cortex in the monkey and chimpanzee, as well as during studies on its projection systems, attempts were constantly made to prove physiologically the existence of those long association tracts joining remote regions of the cerebral hemispheres which have been described in the human brain. Several have been found but, as they belong in no case to any one of the anatomical or functional subdivisions of the hemisphere, it was thought best to report them in a separate article. The long callosal fibers have already been described (1) and will not be herein mentioned, except for the interhemispheric connections of area 18.

## METHOD

The method of physiological neuronography has been explained in previous articles (2). All experiments were performed under full Dial anaesthesia, with the brain exceptionally widely exposed and with the pia mater and cerebral blood supply and drainage as nearly intact as possible. Electrodes were placed, sometimes at one site and sometimes at another, so that practically all parts, excepting the most medial portion of the inferior surface behind the temporal lobe, had been examined by the end of the experiments. The same procedure was followed with respect to strychninization. This was in each case performed by application of a few square millimeters of filter paper moistened with a saturated solution of strychnine sulphate. The activity was recorded before and after the strychninization. Photographs were made and the positions of electrodes and strychnine recorded on these. After sacrificing the animal the brain was peeled, rephotographed and studied to determine as precisely as possible the sites of electrodes and strychnine and thus to prepare the composite diagrams presented below.

## RESULTS

The experiments disclosed the existence of three long fiber tracts, originating and terminating on the convex surface of the hemisphere: (i) in the chimpanzee, strychninization of the posterior margin of band I, which by stimulation was proved to be the eye field (area 8 of Brodmann) produces well-defined strychnine spikes propagated to area 18 of the same hemisphere, and also to the corresponding area of the opposite hemisphere [see (5), Fig. 2]. By partial strychninization of the ipsilateral area 18 during this strychninization of band I, it was possible to obtain a relaying of the dis-

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turbance of the ipsilateral area 18 to the contralateral. The time of this relayed spike is such that it follows the direct spike by more than an entire spike duration—that is, by more than 50 msec. (Fig. 1). This is considered worth reporting because of the strength of the commissural connections be-

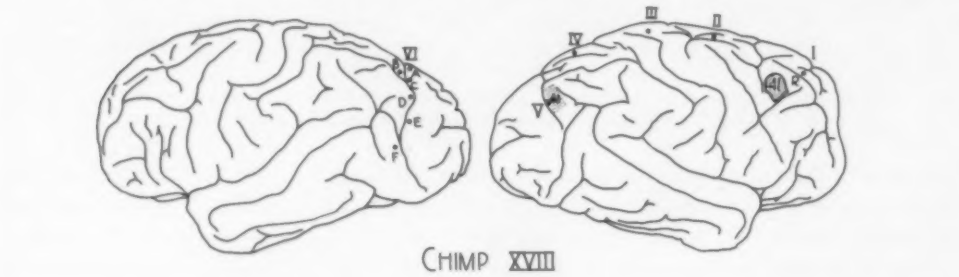


FIG. 1. CHIMP. XVIII. Strychninization 41. The outline sketch of the brain shows location of strychnine 41, location of previous strychnine 40, and location of sixth row of electrodes on the contralateral hemisphere. The spikes from strychnine 40 are now set off by the small spikes from strychnine 41. The top tracing is from the rover (*R*) at a gain of 8 $H_2$. The next four tracings are from cortical leads, *ab*, *bc*, *cd*, and *de* of row six on the contralateral hemisphere, at a gain of 4 $H_2$ (some 60 cycle disturbance in *cd* contralateral lead). The last tracing is from electrode V of the ipsilateral hemisphere, at a gain of 8 $H_2$, and shows small direct spikes from stychnine 41 followed by large spikes which they provoke from the partial residual strychninization of 40. At the right hand is a spike from strychnine 41 transmitted to contralateral *cd;* at the center a spike from strychnine 40 which fires contralateral *cd;* at the left hand both spikes fire into *cd*. Preceding each spike at V from strychnine 40 is a smaller disturbance transmitted from the strychnine 41. Paper speed 6 cm/sec.; tracing reduced 59 per cent.

tween the area 18. (ii) Strychninization anywhere in area 18, whether on the medial, lateral or inferior surface of the hemisphere, results in strychnine spikes propagated to some part of the inferior temporal convolution (area 20) (3). In general, those from the more inferior portion of area 18 are propagated further toward the tip of the temporal lobe. On several occasions

this disturbance was found to extend just onto the central portion of the upper lip of the second temporal sulcus; but at no time could these disturbances be traced to the extreme tip of the temporal lobe, either in the monkey or in the chimpanzee. Figure 2 exemplifies these findings. (iii) Strychninization of the area orbitalis agranularis (area 47 of Brodmann or

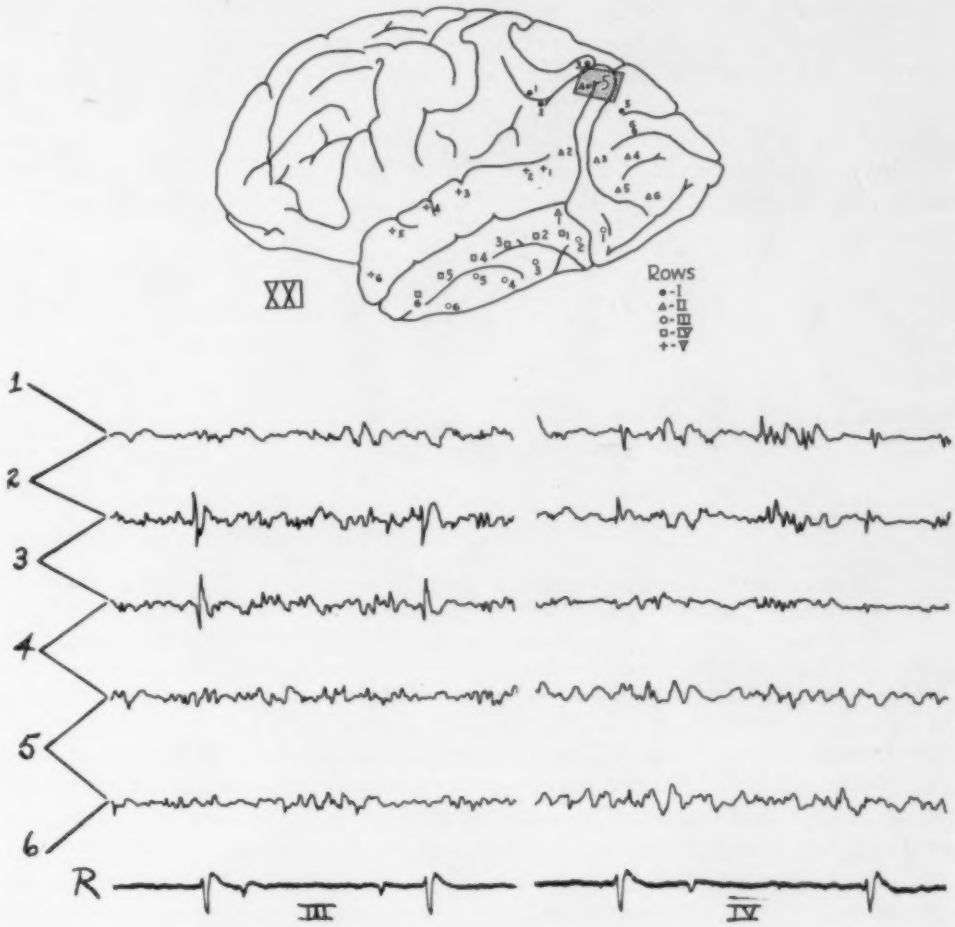


FIG. 2. CHIMP. XXI. Strychninization 5. Left hemisphere. Diagram shows localisation of strychnine 5 and position of electrodes. The tracings are of rows III and IV. Firing in 3 of row IV and in 1, 2 and 3 (feebly) of row III. Rover at the bottom.

FFA of v. Economo in the human brain) produces well-defined strychnine spikes propagated to the anterior portion of the temporal lobe—that is, to what Brodmann calls area 38 in his map of the human cortex. Figure 3 exemplifies this.

The negative findings are equally important. First, at no time were any strychnine spikes propagated in the reverse direction between the areas in-

dicated. Finally, no other tracts were found connecting equally remote portions of the hemisphere.*

## Discussion

The method of local application of strychnine and recording action potentials has been shown to disclose not only the existence but also the direction of the fibers between the gray masses of the brain; for strychnine acts only where axonal terminations contact cell bodies and causes disturbances propagated in the normal direction, not antidromically. Thus it was reasonable to expect that its application to the problem of the direction of the long associational tracts of the cortex would help to show in what areas these systems originated and terminated. Burdach, (1819–1826), Arnold (1838) and others, by dissection of the human brain, discovered four main associational bundles

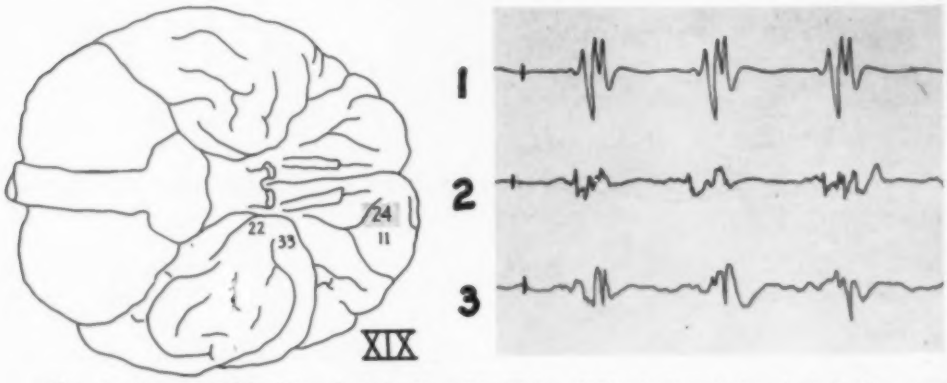


Fig. 3. Chimp. XIX. Strychninization 24. Firing (11) at site of strychninization and (22) and (33) at tip of temporal lobe. Bipolar recording. Sketch of the ventral surface of the brain shows area strychninized and location of electrodes.

but, because of obvious deficiencies of the method, had not been able to determine accurately their origins or terminations. Subsequent anatomists have usually been content with schematic representations, one of which, ascribed by Tilney and Riley to Meynert, is given below (Fig. 4). It was a matter of surprise that each of the three associational bundles herein reported was found to be unidirectional in its transmission, and to arise and to terminate in a functionally and cytoarchitectonically unique area of the cerebral cortex, as indicated in Fig. 4.

In addition to the long longitudinal fiber systems others, running in a transverse direction, have been described in the human cerebral hemisphere. Those described within the occipital cortex—vertical occipital fasciculus of Wernicke, stratum proprium cunei of Sachs, transverse occipital fasciculus of the lingual lobule of Vialet—are evidently, as was shown by v. Bonin,

* In recent experiments on the medial surface of the hemisphere exceptions to this statement have been found.

Garol and McCulloch (4), fibers connecting various parts of the parastriate area. In general these are not as distinct bundles as are the longitudinal fasciculi. It might be pointed out here that we have found abundant evidence of such long transverse fibers in the firing of entire vertical bands on the convexity of the sensory cortex of the chimpanzee, but rarely of a definite fasciculus arising in one cytoarchitectonic area and terminating in another remote area. At least one such exists, however, in the parietal area of the chimpanzee. It extends from just above the interparietal sulcus to an area just above the posterior part of the Sylvian fissure.

Special emphasis should be laid on the time relations, for the conduction in each of these associational bundles occurs with axonal velocities. In fact, it requires high paper-speed to distinguish on an inkwriter record which

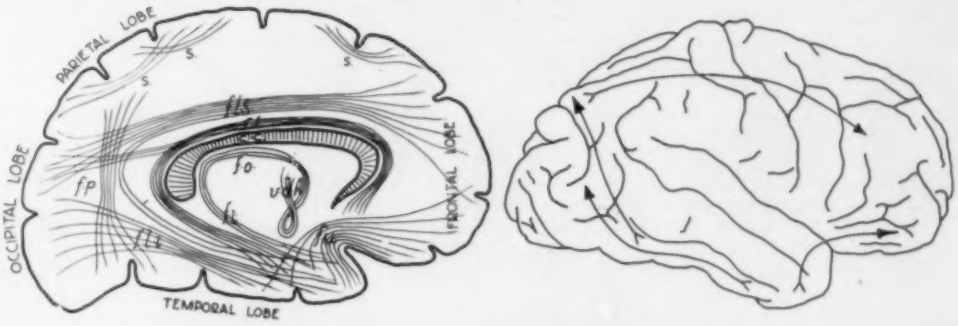


FIG. 4. *Left:* diagram of long fiber tracts in human brain according to Meynert. Seen from medial surface. *Right:* diagram of longest fiber tracts in chimpanzee brain as determined by strychninization and electrical recording.

strychnine spike occurs first—*i.e.*, the one in the propagating area or the one in the recipient area. The contrast between the rapidity of these disturbances and those which are relayed—*i.e.*, are post-synaptic—is brought out by the experiment in which the strychnine spike was conducted the long distance from area 8—*i.e.*, band I—to both ipsi- and contralateral areas 18 at high velocity; whereas the relayed spike crossing from one area 18 to another, after partial strychninization of one of these areas, is delayed so long that the last traces of the axonal termination spike has subsided—in a matter of more than 50 msec. (Fig. 1).

Consideration of the functional organization of the primate cortex as a whole might suggest the existence of a pathway from the temporal lobe to remote regions of the cortex, but the present experiments have failed to reveal any such structure.

## SUMMARY

By applying strychnine locally to the cerebral cortex of the monkey and chimpanzee, and recording the electrical activity, the origin and termination of homologues of three of the well-defined long association bundles of the

human cerebral cortex have been disclosed. From area 8, the frontal suppressor area, there arises a tract leading to area 18, the parastriate area; this is probably part of the superior longitudinal fasciculus of Burdach. From area 18 there arises a tract leading to area 20, on the inferior temporal convolution; it is usually called the fasciculus longitudinalis inferior, and may comprise also what anatomically has been called the vertical occipital fasciculus of Wernicke. There is a tract arising from the area orbitalis agranularis (called area 47 in the human brain) passing to the tip of the temporal lobe (called area 38 in the human brain); it is ordinarily called the fasciculus uncinatus. Each of these pathways normally conducts in one direction only.

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# THE EFFECT OF OXYGEN TENSION ON THE METABOLISM OF CEREBRAL CORTEX, MEDULLA AND SPINAL CORD

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WHEN THE supply of oxygen to the central nervous system falls progressively, normal activity diminishes and irreversible changes occur. For many years differences in response to low oxygen tension have been recognized between cortex, medulla, and spinal cord. The purpose of this study is to investigate possible metabolic bases for these differences.

## METHODS

Twenty-five cats were employed in these experiments. Although full grown animals were not always available, those as small as 1.1 kg. did not differ from the adult. Following painless sacrifice, a sagittal cut through the head was made with a guillotine, permitting immediate exposure of the brain. At room temperature (about 25°C.) slices were cut from the outer surface of the cerebral cortex, from the medulla oblongata in parasagittal plane, and from the cervical portion of the spinal cord transversely. Oxygen uptake at 38° was measured by the single vessel method of Warburg (16) in vessels of 11 cc. capacity containing 2 cc. of medium. The medium contained 0.118 M NaCl, 0.0024 M KCl, 0.0017 M $CaCl_2$, 0.00066 M $MgCl_2$, 0.003 M $NaH_2PO_4$, 0.017 M $Na_2HPO_4$ and 0.011 M dextrose. A high rate of shaking was employed, 160 cycles per min. through 1.5 cm. The first reading was taken about 30 min. after decapitation, including from 10 to 15 min. in the bath for gassing and equilibration; readings were continued for 2 hours. The results are reported on the basis of dry weight of tissue at the end of the experiment. This was of the order of 10, 20 and 35 mg. for cortex, medulla and cord respectively. With cord, and to a lesser extent with medulla, the slices sometimes partially disintegrated; the residuum was centrifuged to remove the medium, and weighed separately. This procedure neglects the weight of the precipitated calcium phosphate, about 0.9 mg./cc. The gas mixtures were made from oxygen and nitrogen (the nitrogen contained from 0.3 to 0.5 per cent oxygen) by displacement of saturated $CaCl_2$ solution from 20 liter carboys; they were analyzed with the Haldane apparatus. Since at 38° water vapor forms 7 per cent of the gas mixture, the oxygen tensions reported in the paper must be corrected by the factor 0.93. Accordingly "100 per cent oxygen" refers to a tension of 0.93 atmosphere. After the removal of the slices the lactic acid in the medium was determined by the colorimetric method of Barker and Summerson (3). Separate determinations for the preliminary period before the first reading were not performed in each case, but a representative series of values is given in Table 1.

## RESULTS

For the most part the rate of oxygen uptake was constant over the two hour period; one consistent variation in rate was the increase from the first to the second hour at the lowest oxygen tensions. Average values for oxygen uptake in oxygen, and lactic acid output at different oxygen tensions are compared in Fig. 1. The ratio, cortex : medulla : cord, was 100 : 34 : 12 for respiration in oxygen and 100 : 17 : 5 for anaerobic glycolysis.

* Aided by a grant from the Milton Fund of Harvard University.

All three tissues were markedly affected by oxygen tension in the range below 21 per cent. The difference between 100 and 21 per cent was significant for the quantity of data obtained only in cortex. When the oxygen uptake was expressed as a percentage of the value for 100 per cent oxygen (Fig. 2), there was no striking difference between the three tissues in the influence of

*Table 1. Lactic acid output during the preliminary period of gassing and equilibration.*

| Exp. | Oxygen mixture | Lactic acid output | | | Manometric equivalent* |
|---|---|---|---|---|---|
| | | (1) Total | (2) Blank | (3) Net for 2 hr. | $Q_G$ (4) (4) = (3)/8 |
| | Vol. per cent | mg./gram tissue | | | mm.³$CO_2$/mg. tissue/hr. |
| **Cortex** | | | | | |
| 216 | 21 | 39 | 17 | 22 | 2.8 |
| 227 | 0.5 | 114 | 13 | 101 | 12.5 |
| 303 | 100 | 69 | 23 | 46 | 5.8 |
| | 3 | 140 | 34 | 106 | 13.3 |
| | 0.5 | 152 | 29 | 123 | 15.4 |
| 316 | 100 | 50 | 11 | 39 | 4.9 |
| 317 | 0.5 | 116 | 16 | 100 | 12.5 |
| 318 | 21 | 86 | 21 | 65 | 8.1 |
| **Medulla** | | | | | |
| 226 | 0.5 | 20 | 3 | 17 | 2.1 |
| 304 | 100 | 24 | 6 | 18 | 2.3 |
| | 3 | 29 | 6 | 23 | 2.9 |
| | 0.5 | 18 | 5 | 13 | 1.6 |
| **Cord** | | | | | |
| 225 | 0.5 | 3 | 0 | 3 | 0.4 |
| 228 | 0.5 | 4 | 1 | 3 | 0.4 |
| 307 | 100 | 10 | 4 | 6 | 0.8 |
| | 3 | 10 | 3 | 7 | 0.9 |
| | 0.5 | 8 | 2 | 6 | 0.8 |

* 1 mm.³ = 0.004 mg. lactic acid.

oxygen tension on oxygen uptake. At most points there was considerable overlapping from one tissue to another.

The effect of oxygen tension on glycolysis was quite different from one tissue to another (see Fig. 1). In cerebral cortex, as the oxygen tension was lowered there was a progressive increase in glycolysis which was proportional to the decrease in oxygen uptake (Fig. 3). The effect was much the same in medulla in the range down to 3 per cent oxygen. From that point instead of

rising to a maximum in nitrogen, however, the glycolysis in this tissue fell. In cord, on the other hand, the effect of oxygen tension was slight.

## Discussion

The rate of oxygen uptake is the metabolic factor commonly discussed in connection with differences between cerebral cortex, medulla oblongata and spinal cord in sensitivity to anoxia. Recent studies of the metabolism of the brain in the growing mammal (9, 15) have confirmed earlier observations that in the adult, the regions less vulnerable to hypoxia have the lower metabolic rate. Comparison of the rates of respiration and of anaerobic

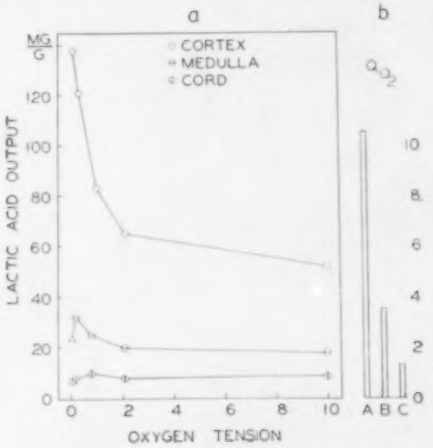


Fig. 1. (a) Data from table 2; column 7 vs. column 1; oxygen tension in atmospheres ×0.93. (b) Mean oxygen uptake in oxygen in mm.³/mg./hr. where A, B, and C refer to cortex, medulla and cord respectively.

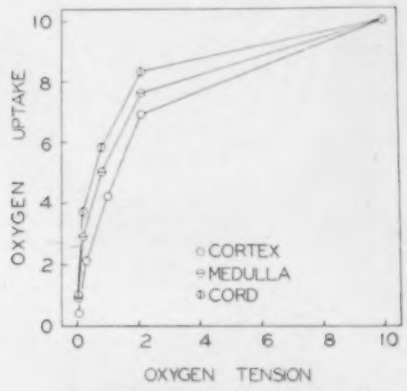


Fig. 2. Data from table 2; column 5 vs. column 1; oxygen tension in atmospheres ×0.93.

glycolysis in the cat, however, suggests that glycolysis may be a factor of some importance (Table 2). The $Q_{O_2}$ of cortex in oxygen was three times as great as that of medulla, while the anaerobic glycolysis of cortex was *six* times that of medulla. This proportionately greater rate of glycolysis of cortex might affect the sensitivity of cortex to anoxia through depletion of glucose or accumulation of acid. Even as the medulla oblongata has an advantage in this regard over cerebral cortex, so the spinal cord appears to have an advantage over medulla when one compares aerobic and anaerobic glycolysis. Oxygen tension had relatively little effect on glycolysis in cord, whereas in medulla glycolysis was 28 per cent higher in nitrogen than in oxygen. The relatively high aerobic glycolysis in cerebral cortex has already been discussed (5). Glycolysis in the medulla has been subjected to little if any direct study since that of Haldi, Ward and Woo (8), and their results

have been questioned by Avery, Kerr and Ghantus (2). The peculiar effect of oxygen tension on glycolysis in the medulla is of significant magnitude, and is controlled technically by the results obtained with cortex.*

We have as yet no definite indication of a mechanism that can explain how glycolysis in the medulla could be maximal at an oxygen tension of from

*Table 2. Means and standard errors of the means.*

| $pO_2$ | Oxygen uptake | | | | Lactic Acid output in 2 hr. + preliminary period | |
|---|---|---|---|---|---|---|
| | | 1st hr. | 2nd hr. | Mean | | |
| Vol. per cent dry | No. of cats | $Q_{O_2}$ = mm.³/mg./hr. | | Per cent of value in 100 per cent $O_2$ | No. of cats | mg./g. |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| | | | Cortex | | | |
| 100 | 15 | 10.5 ±0.35 | 10.2 ±0.27 | 100 | 14 | 52 ± 3.9 |
| 21 | 10 | 6.7 ±0.39 | 7.1 ±0.32 | 69 ± 5.3 | 9 | 65 ± 6.4 |
| 10 | 8 | 3.9 ±0.48 | 4.5 ±0.40 | 42 ± 5.4 | 7 | 83 ± 6.2 |
| 3 | 9 | 2.0 ±0.11 | 2.4 ±0.10 | 21 ± 1.4 | 7 | 121 ±12.7 |
| 0.5 | 9 | 0.1 ±0.12 | 0.7 ±0.13 | 4 ± 0.8 | 7 | 138 ±14.5 |
| | | | Medulla | | | |
| 100 | 14 | 3.5 ±0.18 | 3.4 ±0.18 | 100 | 14 | 18 ± 2.2 |
| 21 | 6 | 2.3 ±0.22 | 2.3 ±0.19 | 76 ±11.6 | 6 | 20 ± 3.3 |
| 8 | 7 | 1.3 ±0.22 | 1.6 ±0.21 | 50 ±10.6 | 7 | 25 ± 2.5 |
| 2 | 7 | 0.9 ±0.14 | 1.0 ±0.14 | 29 ± 5.8 | 7 | 32 ± 3.1 |
| 0.4 | 7 | 0.2 ±0.09 | 0.4 ±0.08 | 9 ± 2.3 | 8 | 23 ± 2.2 |
| | | | Cord | | | |
| 100 | 12 | 1.3 ±0.10 | 1.3 ±0.07 | 100 | 12 | 9 ± 1.0 |
| 21 | 6 | 1.0 ±0.16 | 1.0 ±0.17 | 83 ±10.7 | 6 | 8 ± 2.1 |
| 8 | 6 | 0.6 ±0.03 | 0.6 ±0.04 | 58 ± 6.8 | 6 | 10 ± 1.8 |
| 2 | 6 | 0.4 ±0.06 | 0.4 ±0.05 | 37 ± 3.9 | 6 | 8 ± 1.4 |
| 0.4 | 5 | 0.0 ±0.10 | 0.2 ±0.03 | 10 ± 4.5 | 6 | 7 ± 1.1 |

2 to 3 per cent of an atmosphere instead of zero. Nevertheless, the observation is of interest in connection with possible explanations of the delayed post-apneic hyperpnea (11), in dogs under light anesthesia deprived of their peripheral chemoreceptors. Evidently the motor impulses arising from the respiratory center pass through a maximum after the readmission of air to

* The following observations should be borne in mind, although they do not contribute directly to an explanation of the effect in medulla. Rosenthal and Lasnitski (12) noted an increase in anaerobic glycolysis of liver and other tissues following an incubation period in oxygen. Ashford and Dixon (1) studied the metabolism of brain cortex in a medium to which M/10 KCl had been added and found an increase in aerobic glycolysis but a decrease in anaerobic glycolysis.

the dogs. Glycolysis in the medulla follows a parallel course. This possible relationship needs to be investigated further, since it may well provide a better understanding of the problems involved in chemical stimulation of respiration.

The oxygen tension-oxygen uptake curves are described by the equation of Tang (14), $P/A = K_1 + K_2P$, where $P$ is oxygen tension, $A$ the rate of respiration, $K_1$ the intercept and $K_2$ the slope. When $P/A$ was plotted against $P$ (Fig. 4), linear relationships were obtained. Tang cited 17 vari-

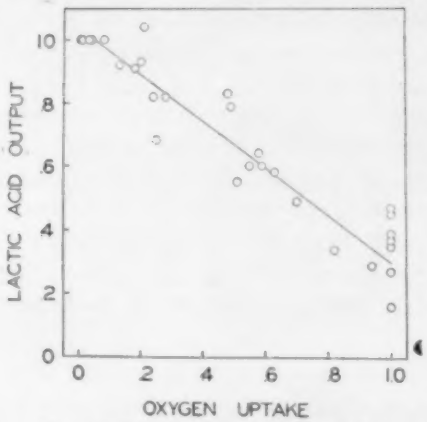


FIG. 3. Cerebral cortex. Data from 7 cats; tissue from each animal was studied at different oxygen tensions. Each point represents the oxygen uptake at an intermediate oxygen tension as a fraction of the oxygen uptake in oxygen for that animal, and the lactic acid output at the same oxygen tension as a fraction of the lactic acid output in nitrogen.

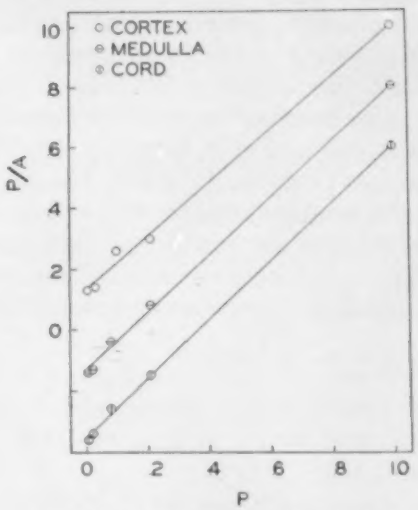


FIG. 4. Data from table 2; A from column 5, P from column 1; oxygen tension in atmospheres ×0.93. The intercepts on the P/A axis were 0.13, 0.07 and 0.05 for cortex, medulla and cord respectively.

eties of biological material the data for which were described by this equation. The curves for brain *in vitro* (Fig. 2) represent a possible deviation from the behavior of brain *in vivo*. In cerebral cortex, oxygen uptake was depressed significantly in 21 per cent oxygen as compared with 100 per cent, whereas *in vivo*, changes in metabolic and mental activity are not observed until the oxygen tension in the inspired air is lower (4). The discrepancy between the $Q_{O_2}$ of cortex *in vivo* and *in vitro* has been discussed elsewhere (5).

Elliott and Libet (7) in studying the oxygen uptake of rat brain in air and in oxygen, found that the substitution of oxygen for air had no effect on the initial rate of a homogenized suspension of whole brain, whereas with slices of cortex there was a 42 per cent increase. This agrees with our figure of 45 per cent for cortex (Table 2.)* They attributed the difference between

* This appears to be true also of medulla and cord but since the differences in 100 and 21 per cent oxygen are smaller than in cortex, a larger body of data would be required to establish the point for them.

the behavior of slices and suspensions to inadequate diffusion of oxygen into the slice. If diffusion into the slice accounts for the high critical oxygen tension shown in Fig. 2, then the data for 100 per cent oxygen are probably most nearly comparable to the situation *in vivo* when air is being breathed, for glycolysis is at a minimum in oxygen *in vitro* and in air *in vivo*. If, on the other hand, Fig. 2 is interpreted to mean that *in vivo* brain metabolism could increase, if a greater partial pressure of oxygen were supplied in the inspired air, the results are significant in a consideration of the toxic action of high concentrations of oxygen. Using a medium free of Ca and Mg ions Elliott and Libet (7) described a decline in rate with time that was more marked in oxygen than in air. In our medium a significant decline in the rate from the first to the second hour (Table 2) was not observed.

The intimate connection between respiration and glycolysis in cortex is illustrated in Fig. 3, for when oxygen tension varies, the relationship between the two processes is linear. The implication of this is to limit the generality of the results obtained with retina in bicarbonate medium (10, 13), in which the Pasteur enzyme appeared to influence glycolysis but not respiration. The relationship between respiration and glycolysis observed in cerebral cortex is also found in bone marrow (17), and in retina in phosphate medium (6).

## Summary

The rates of oxygen uptake of cortex, medulla and spinal cord were in the ratio 100:34:12, whereas the ratio for anaerobic lactic acid production was 100:17:5.

The oxygen uptake of all three tissues was sensitive to oxygen tension. The shape of the oxygen uptake-oxygen tension curve was essentially the same for the three tissues.

The anaerobic lactic acid production was twice as great in cortex as in medulla per unit of oxygen uptake in oxygen. Lactic acid production was sensitive to oxygen tension in cortex and medulla but not in cord. These differences in lactic acid production are offered in partial explanation for the variations in resistance to anoxia from one region to another within the central nervous system.

In cortex, lactic acid production and oxygen uptake varied inversely when the oxygen tension was altered.

In medulla, surprisingly enough, lactic acid production was maximal at 2 to 3 volumes per cent oxygen. The possible relationship of this to a previously described respiratory phenomenon was mentioned.

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# THE INTERACTION OF ANTIDROMIC AND ORTHODROMIC VOLLEYS IN A SEGMENTAL SPINAL MOTOR NUCLEUS

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A MAXIMAL antidromic volley (1) backfired into a segmental spinal pool of motoneurons fails to block a reflex volley completely unless the opposed volleys clash in the motor axons. The following experiments describe, and attempt to account for, this finding.

In Fig. 1 are presented the results of an experiment in which an antidromic volley and an orthodromic (reflex) volley pertaining to two-neuron-arc reflex systems are caused to interact. The reflex of the first sacral segment is utilized in this experiment, as in most of the others, for the ventral root of this segment in the cat is of satisfactory length for the placing of the required electrodes. Record 1A illustrates the reflex volley recorded in isolation from the ventral root. In records 1B–1T, antidromic and reflex volleys are combined, the orthodromic shock initially preceding and subsequently trailing the antidromic shock. The orthodromic shock remains fixed on the time axis of the successive records.

When the orthodromic shock precedes the antidromic shock by 0.75 msec. (0–0.75–A) as in Fig. 1B, the orthodromic response is completely blocked, as it is with the use of greater stimulus intervals until the orthodromic response begins to reach the recording leads before the initiation of the antidromic volley. A response to the orthodromic shock is first secured in Fig. 1C, recorded with a shock separation of (0–0.6–A). The orthodromic response increases until (A–0.1–0) in record 1F, following which it remains constant until (A–0.9–0) in record 1J, after which a second rise in amplitude is realized.

In earlier experiments employing antidromic conditioning (1, 5, 8) the first orthodromic responses were obtained with shock separations of (A–0.43–0) to (A–2.3–0), which would correspond approximately to records J and O in the series of Fig. 1. The initial step in recovery, therefore, has not been described previously.

Figure 2, curve A, illustrates graphically another experiment similar to that presented in Fig. 1. On theoretical grounds the ideal zero for a recovery curve employing synaptic excitation would be that at which the antidromic volley coursing over the somata of the motoneurons (6) reaches the points at which the postsynaptic reflex responses are evoked at the exact time that the orthodromic volley reaches those points. One can appreciate that the ideal zero is not readily attainable in practice. However, a useful correction can be made by allowing for conduction in the motoneuron axons, which can be measured satisfactorily. Separate measurements are required for the ventral root conduction time of the reflex and antidromic volleys, as these

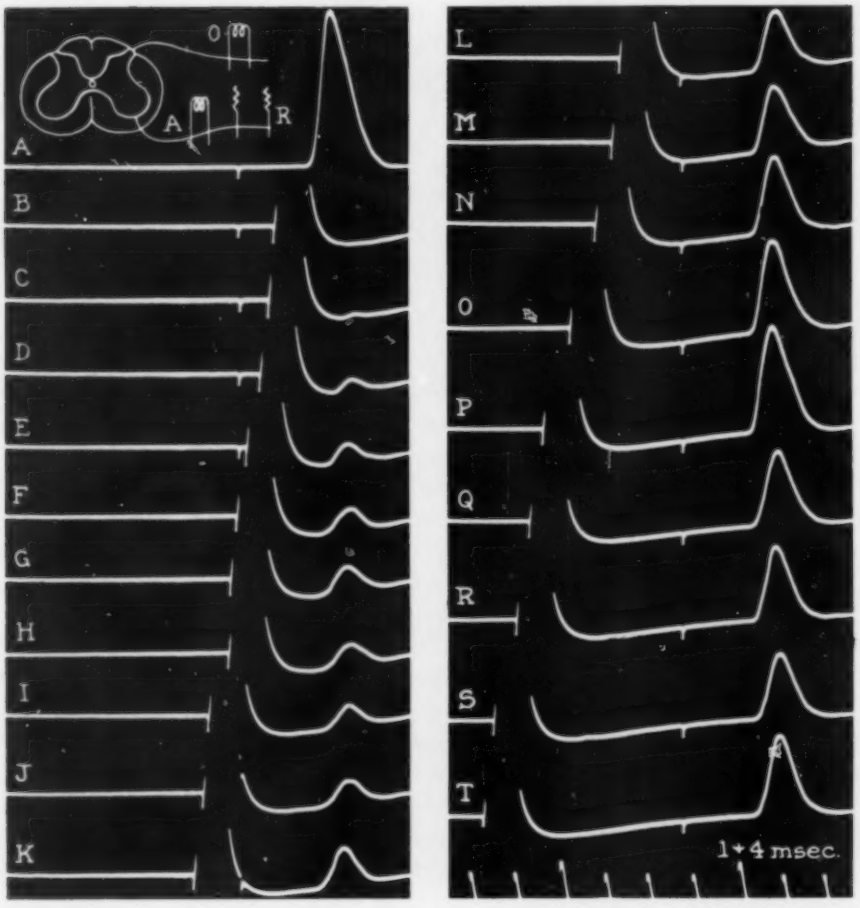


FIG. 1. Segmental (S1) reflex preparation. The recovery of reflex response following an antidromic volley. The inset serves to illustrate the disposition of stimulating and recording electrodes. The orthodromic, or reflex, volley is evoked by stimulation through electrodes O. The antidromic volley is evoked by stimulation through electrodes A on the ventral root. Records are obtained by means of electrodes R on the ventral root distal to the A electrodes. Record A: reflex response in isolation. Records B–T: reflex response with combined orthodromic and antidromic stimulation. The antidromic shock falls progressively earlier with respect to the orthodromic shock which is fixed in position on the sweep. Note two stage recovery with "plateau" period (records F–J) during which the reflex remains relatively constant in size. Time in 1 and 4 msec. intervals.

times differ slightly due to the different position of the stimulating and recording leads on the ventral root.

Ventral root conduction time for the antidromic volley is obtained by stimulating with the A electrodes (Fig. 1, inset) and recording the motoneuron response by means of a microelectrode, the interval between the A shock and the onset of negativity at the microelectrode denoting the value for the correction. Ventral root conduction time for the orthodromic volley

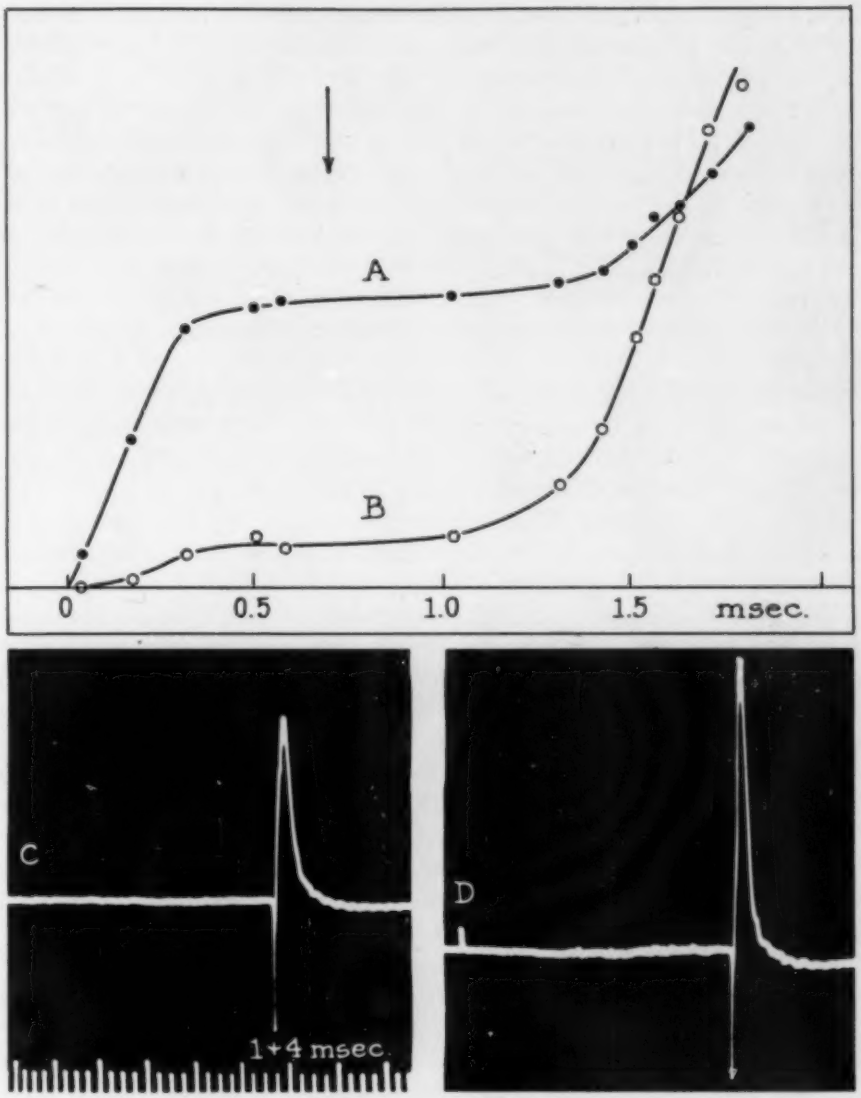


FIG. 2. A: recovery curve constructed from observations such as those presented in Fig. 1. Ordinate—amplitude of the reflex response in arbitrary units. Abscissa—time. The arrow indicates coincidence of the antidromic and orthodromic shocks. To the left of the arrow the orthodromic shock leads; to the right of the arrow the antidromic shock leads. Time is plotted according to the conduction corrections explained in the text. B: recovery curve constructed in the same manner as curve A. All details for curves A and B are identical with the single exception that the antidromic volley employed for the observations plotted in curve B was preceded by a single shock to the ipsilateral brachial plexus. Note reduction of the initial recovery stage. C: potential recorded by microelectrode from the motoneurons of the S1 segment as the consequence of a single maximal antidromic volley. D: as in C except that the antidromic shock is preceded by a single shock to the ipsilateral brachial plexus with the result that the soma response (7, 8) of the motoneurons is facilitated. Time for C and D below record C in 1 and 4 msec. intervals.

is obtained by inserting stimulating electrodes into the ventral horn in such a way that the motoneurons may be stimulated directly by an electrical shock, the response being recorded by means of the R electrodes. When the orthodromic shock antecedes the antidromic shock by an interval equivalent to the total reflex latency minus the sum of the ventral root corrections, the opposed volleys should clash at the junction of axon and soma of the motoneurons. Zero on the scale of ordinates is set so as to represent this interval of shocks. In consequence the time scale represents, within the limits of measurement, the interval between the arrival of the antidromic volley and the arrival of the orthodromic volley at the axon-soma junctions. The arrow indicates the status at the coincidence of the two shocks, orthodromic and antidromic.

When the antidromic and orthodromic volleys coincide at the axon-soma junction, as indicated by the origin of curve 2A, the orthodromic volley is obliterated. The orthodromic volley, however, need reach the junction only 0.1 msec. later than the antidromic volley to be successful in eluding to some extent total refractoriness. The orthodromic volley, by the time it reaches the axons of the motoneurons, has been subjected to considerable conduction and to synaptic transmission, with the certain result that it is more dispersed than the antidromic volley arriving at the same point. In consequence it may be that only the last impulses of the dispersed orthodromic volley to arrive at the axon elude refractoriness to reach the recording leads when such short intervals are employed. Nevertheless it is apparent that the orthodromic volley passing through the reflex arc is capable of exciting the motoneuron axon for a period approximately equal in duration to the absolutely refractory period of the axon. The added delay in the reflex response, which is easily seen in Fig. 1, presumably is due to refractoriness and takes place at the axon junction. As the reflex volley falls later the response increases sharply to a plateau which is maintained for approximately 1 msec. before a further increase in the response takes place.

The observations of Fig. 1 and 2A indicate that some impulses in the orthodromic volley are not blocked by the antidromic volley when the two volleys meet in the motoneurons at some point central to the axon junction, even though the antidromic volley blocks the orthodromic volley completely when they meet in the axons of the motoneurons. In attempting to account for these observations it is germane to consider some aspects of the response of the motoneuron somata to antidromic activation.

The action potential of the motoneurons, excited by antidromic shocks and recorded by the use of a microelectrode appropriately situated, consists of an initial positive deflection signalling the approach of the volley in the axons, followed by a large negative deflection, this in turn being followed by a more prolonged positive deflection (6). The motoneuron potential as recorded in the sacral spinal cord may be seen in Fig. 2C. The large negative "spike" potential of the motoneuron somata, in contrast to the spike potential of the axons, is labile (7); it is easily blocked by asphyxia (7); it is pro-

gressively reduced on tetanic stimulation at frequencies which the axons follow with ease; it may be facilitated and inhibited (10).

The fact that the soma response to a maximal antidromic volley may be facilitated indicates that the antidromic volley fails to enter the somata of some of the motoneurons or that, on entering, it produces effects graded in extent or intensity. Of these possibilities, the first, failure of the antidromic volley to extend beyond the axons into the somata of some of the motoneurons available to the orthodromic reflex volley, would account for the initial rise and plateau of the recovery experiments illustrated in Fig. 1 and 2, for in these motoneurons the orthodromic volley would not encounter refractoriness until reaching the axon junction. An explanation of the initial stage in recovery based on the preceding considerations may be put to test by the simple expedient of facilitating or depressing the response of the motoneuron somata to the conditioning antidromic volley.

In order to compare the recovery curve of reflex responses in association with resting and facilitated antidromic volleys it is useful to procure a facilitating background activity which is relatively constant over a period of several milliseconds. A single shock to the brachial plexus (3) provides an admirable source of facilitatory impulses for it satisfies the present requirements. Fig. 2C illustrates the motoneuron potential recorded by means of a microelectrode in the resting spinal cord; the stimulus is a single maximal antidromic shock. Fig. 2D shows how the soma potential is greatly augmented and slightly synchronized by the activity resulting from the brachial plexus stimulation. The initial positive deflection, signalling the approach of the antidromic volley in the axons, is unaltered from record C to record D.

Figure 2, curve A, as noted before, illustrates graphically the initial stage in recovery of reflex response. Curve B is constructed in a similar manner from observations obtained under identical conditions with the single exception that the antidromic volley is preceded, as in 2D, by a single shock to the brachial plexus. A comparison of curves A and B reveals that the size of the reflex responses is severely reduced for the duration of the initial stage or plateau. Subsequently the action of the brachial plexus stimulation is to facilitate the reflex volley as it does in the absence of the antidromic volley (3), the reflex volley, of course, still being of subnormal size. Thus the greater the soma response to the antidromic volley the less spectacular are the reflex responses of the initial stage in recovery.

If the reduction in size of the initial stage reflex responses illustrated in Fig. 2 is indeed due to increase in the response of the motoneuron somata to the antidromic volley, then it follows that the initial stage reflex discharges should increase in size as the response of the motoneuron somata to the antidromic volley is depressed. Depression of the soma response of the motoneurons may be attained by repetitive antidromic stimulation (10). Before discussing the effects of repetitive antidromic stimulation it is appropriate to consider another type of motoneuron discharge to the periphery which occurs during the period of central activity following an antidromic shock.

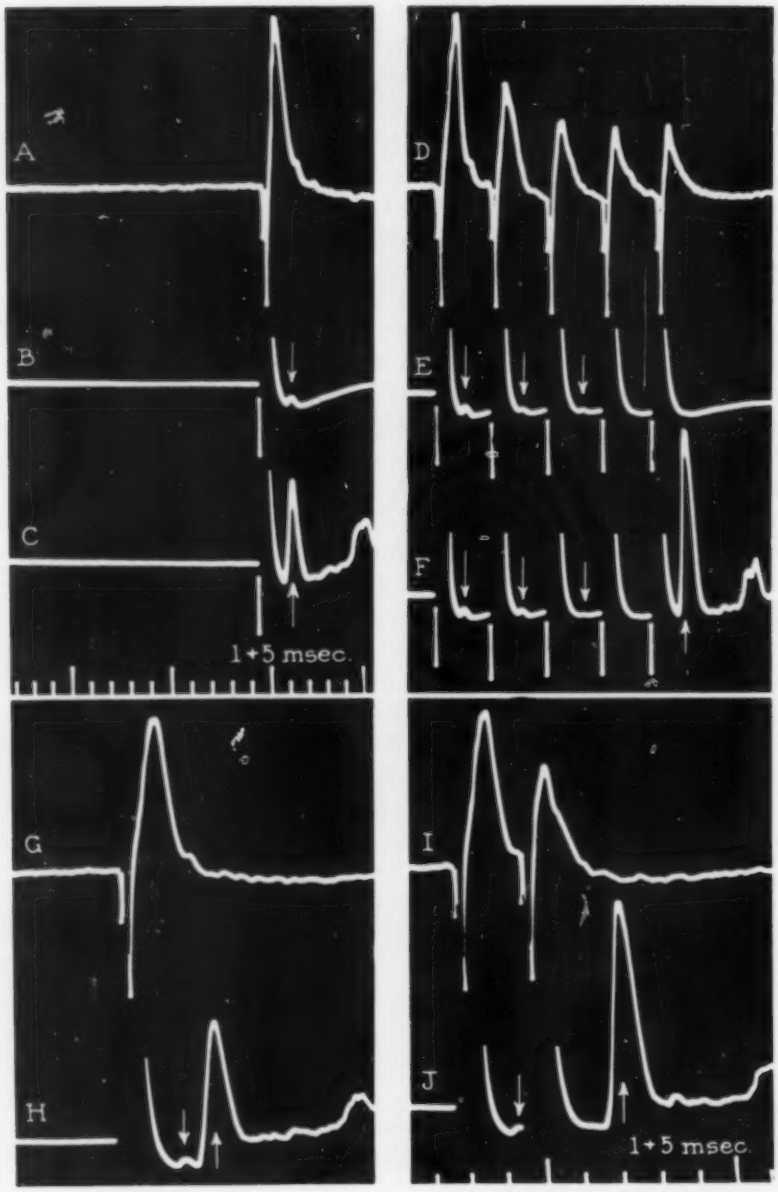


FIG. 3. Changes in the soma response of the motoneurons, in the recurrent motoneuron discharge and in the initial stage reflex discharge resulting from repetitive antidromic stimulation. Recurrent discharges are identified by means of arrows directed downward; initial stage reflex discharges by means of arrows directed upward. Further description in text. Time for records A–F in 1 and 5 msec. intervals is below record C. Time for records G–J in similar units is below record J.

Frequently, among spinal motoneurons at any rate, the transmission of an antidromic volley is followed by the discharge of a small centrifugal volley in the absence of any other specific stimulation (9). The central latency for this "pseudo-reflex" volley, or recurrent discharge as it will be called, is somewhat less than 1 msec. Furthermore the recurrent discharge occurs only in motoneurons that are occupied by the antidromic volley; it may be inhibited by appropriate orthodromic activity. In appearance the recurrent volley is not unlike the initial stage reflex volley, but the two may be differentiated quite easily by experiment.

The observations of Fig. 3 illustrate the behaviour of the initial stage reflex discharge when an orthodromic volley is associated with single and repetitive antidromic volleys. Shown also is the differentiation between recurrent motoneuron discharges and initial stage reflex responses. Record A of Fig. 3 shows the motoneuron potential recorded by a microelectrode and resulting from a single antidromic volley. In 3B is recorded, from the ventral root, the antidromic volley followed closely by the recurrent motoneuron discharge. In Fig. 3C, a single dorsal root shock is delivered synchronously with the antidromic shock. The initial stage reflex discharge may be seen in 3C occupying the same relative position as the recurrent discharge in 3B. Fig. 3, A, B, and C form control records for the consideration of 3D, E, and F in which five antidromic volleys are utilized.

Figure 3D records the motoneuron potentials evoked by five rapidly repeated antidromic volleys. It will be seen that the soma potential is progressively reduced (*cf.* 10). On the contrary the initial positive deflection, signalling each volley approaching in the axons toward the microelectrode, retains its maximal size throughout. In 3E is shown the result of five antidromic volleys as recorded on the ventral root. By comparing 3E with 3D it will be seen that the recurrent motoneuron discharges decrease in parallel with the soma potentials and reach final extinction. Examination of 3F, however, shows that the initial stage reflex discharge is differently affected. In 3F, as in 3C, a dorsal root shock is delivered simultaneously with an antidromic shock, in this case with the final antidromic shock of the series. Again as in 3E, the progressive decrease in the recurrent discharges is evidenced, but the initial stage reflex discharge is greatly increased over its size following a single antidromic volley.

Figure 3 (G–J) repeats the experiment just described with the exception that the orthodromic shock is delivered approximately 0.6 msec. later than the corresponding antidromic shock. By this device the reflex discharge is kept within the initial stage or plateau period, but it is sufficiently late to clear entirely the recurrent motoneuron discharge, thus avoiding any confusion between the two discharges. Records G and I of Fig. 3 illustrate the soma potentials recorded following a single and two antidromic volleys respectively. Comparison of 3H and 3J shows that the recurrent discharge drops out, whereas the initial stage reflex is enhanced as the soma potential is depressed after an antecedent antidromic volley.

*On the question of refractoriness of the motoneuron somata.* The initial stage reflex discharge appears first at the end of absolute refractoriness in the motoneuron axons, or shortly thereafter, and while some, but not all, of the motoneuron somata are still occupied by the antidromic volley. The initial stage reflex discharges are obtained by transmission through those motoneurons in which the antidromic volley presumably is blocked at the axon junction. The early initial maximum and plateau show that within a few tenths of a msec. virtually all of the motoneurons available to the orthodromic volley by reason of the failure of the antidromic volley are recruited to the service of the orthodromic volley. Approximately 1 msec. after the end of absolute refractoriness at the axon, the plateau of the initial stage reflex response ends and a second stage of recovery begins. The second stage of recovery appears to represent the beginning of transmission of the reflex volley through those motoneurons in which the antidromic volley enters the soma. One may conclude that conduction in the motoneuron soma is accompanied by refractoriness, and that on the occasion of antidromic activation the motoneuron is not open to synaptic activation for approximately 1 msec. after the axon has recovered from absolute refractoriness. This statement must not be taken to mean that the refractory period of the soma is longer by 1 msec. than that of the axon, since the upper limit for a refractory period of motoneuron somata has been set at 0.6 msec. (4) which is virtually that of the axons.

*Other considerations.* If the antidromic impulse dies at the axon-soma junction of some motoneurons (*cf.* 7), which appears to be the case, then one might expect the same changes to take place in the somata of those motoneurons as are known to take place beyond a block in nerve (2, 6). On this supposition the somata of the motoneurons in which the antidromic volley dies would be more excitable than in the "resting" state and would accordingly be more accessible to the reflex volley than they would be in the absence of the antidromic volley. On the other hand the activity in the fully occupied motoneurons might affect adversely the transmission of impulses in the orthodromic direction through the motoneurons that are not fully occupied by the antidromic volley (9). Considerations such as these give some hint of the complexity of the central disturbance created by an antidromic volley. In general it seems that antidromic volleys should be employed with due caution, and that oversimplified assumptions as to the central effect of an antidromic volley should be avoided. There can be little question but that an antidromic volley does more than interpose a refractory period in the path of a reflex.

## Summary

A maximal antidromic volley backfired into a segmental spinal pool of motoneurons fails to block a reflex volley completely unless the opposed volleys clash in the motor axons. This is apparently due to the failure of the antidromic volley to conduct from the axon into the soma in some of the

motoneurons. In consequence the recovery curves obtained by the use of synaptic stimulation reveal two stages, the first rise being referable to axonal recovery of their motoneurons the somata of which are not activated by the antidromic volley, the second to recovery of synaptic transmission through the motoneurons in which the antidromic volley does occupy the somata.

The prominence of the initial stage depends upon the number of motoneurons in which the antidromic volley fails to evoke a soma response.

Conduction in the neuron soma is accompanied by refractoriness.

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